A Mouse Model of Serotonin 1B Receptor Modulation of Cocaine and Methamphetamine Craving

by

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ABSTRACT

Serotonin 1B receptors (5-HT_{1B}Rs) are a novel target for developing pharmacological therapies to reduce psychostimulant craving. 5-HT_{1B}Rs are expressed in the mesolimbic pathway projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), which is involved in reward and motivation. 5-HT_{1B}R agonists modulate both cocaine- and methamphetamine-seeking behaviors in rat models of psychostimulant craving. In this dissertation, I tested the central hypothesis that 5-HT_{1B}Rs regulate cocaine and methamphetamine stimulant and rewarding effects in mice. I injected mice daily with cocaine for 20 days and then tested them 20 days after their last injection. The results showed that the 5-HT_{1B}R agonist CP94253 attenuated sensitization of cocaine-induced locomotion and cocaine-seeking behavior, measured as a decrease in the ability of a cocaine priming injection to reinstate extinguished cocaine-conditioned place preference (CPP). Subsequent experiments showed that CP94253 given prior to conditioning sessions had no effect on acquisition of methamphetamine-CPP, a measure of drug reward; however, CP94253 given prior to testing attenuated expression of methamphetamine-CPP, a measure of drug seeking. To examine brain regions and cell types involved in CP94253 attenuation of methamphetamine-seeking, I examined changes in the immediate early gene product, Fos, which is a marker of brain activity involving gene transcription changes. Mice expressing methamphetamine-CPP showed elevated Fos expression in the VTA and basolateral amygdala (BlA), and reduced Fos in the central nucleus of the amygdala (CeA). In mice showing CP94253-induced attenuation of methamphetamine-CPP expression, Fos was increased in the VTA, NAc shell and core, and the dorsal medial caudate-putamen. CP94253 also reversed the

methamphetamine-conditioned decrease in Fos expression in the CeA and the increase in the BlA. In drug-naïve, non-conditioned control mice, CP94253 only increased Fos in the CeA, suggesting that the increases observed in methamphetamine-conditioned mice were due to conditioning rather than an unconditioned effect of CP94253 on Fos expression. In conclusion, 5-HT_{1B}R stimulation attenuates both cocaine and methamphetamine seeking in mice, and that the latter effect may involve normalizing activity in the amygdala and increasing activity in the mesolimbic pathway. These findings further support the potential efficacy of 5-HT_{1B}R agonists as pharmacological interventions for psychostimulant craving in humans.

DEDICATION

To all of my mentors and colleagues over the years,
for inspiring me, challenging me,
and giving me the confidence to achieve my goals.

To my entire family and dear friends,
for providing exceptional support and
unconditional love throughout this journey.

To my mom, dad, and grandma for always
being proud of me and encouraging me
to follow my dreams
and providing me with the tools to succeed.

Finally, I would like to dedicate this dissertation
in memory of the late Neshan Derghazarian, my father,
who loved me so dearly and wanted nothing more
than success and happiness for his family.

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CHAPTER 1

GENERAL OVERVIEW

Drug addiction is a chronic and debilitating condition. Ultimately, addiction leads to psychological, physical, and social impairment and distress. Clinically, addiction is referred to as dependence and is the most severe form of substance use disorders (SUDs). SUDs can be thought of as a spectrum disorder along a continuum ranging from mild to severe. SUDs are characterized by excessive time being preoccupied with the drug or obtaining the drug, using the drug, persistent desire or craving for drug, continued use despite negative consequences, tolerance and withdrawal, followed by shame and guilt. The cost of SUDs is extremely high due to crime, lost work productivity, and health care costs. In 2007, the cost of illicit drug use was $193 billion, not including prescription opioids (Birnbaum et al., 2011; National Drug Threat Assessment, 2011). Prescriptions opioids alone cost society $78.5 billion in 2013 (Florence, Zhou, Luo, & Xu, 2016). In 2010, the cost of alcohol and tobacco use was estimated at $249 billion (Centers for Disease Control and Prevention) - $300 billion (Surgeon General; Xu, Bishop, Kennedy, Simpson, & Pechacek, 2014). Not only is there a huge financial cost to society related to SUDs, but also tremendous personal burden to the individual, their friends, and family.

Another key component of addiction is relapse (Leshner, 1997; O'Brien, Childress, Ehrman, & Robbins, 1998; Wallace 1992). Addiction is often perceived as a cycle of drug dependence with the nature of the disease including drug use, shame/guilt, abstinence, and relapse. Individuals will relapse numerous times while in treatment (McLellan, Lewis, O'Brien, & Kleber, 2000). Triggers of drug relapse during attempted abstinence include stress, anxiety, as well as exposure to drug-associated cues (Koob &

Le Moal, 2005; Sinha 2008). Cues that were previously associated with drug taking, such as paraphernalia, the environment, and the individuals present during the drug experience, can lead to craving and serve as a motivator to seek out the drug. Additionally, individuals may use again in hopes of reducing the withdrawal symptoms they are experiencing or to feel 'normal' again. Therefore, much research is devoted to examining the neural mechanisms underlying the high propensity for relapse. In addition, understanding the rewarding properties of drugs and the incentive motivation to seek drugs is important. It is imperative to find novel therapeutics for SUDs as so many individuals and their families suffer because of this chronic disease.

Drugs of abuse exert their reinforcing effect primarily by activating dopamine neurons that originate in the ventral tegmental area (VTA) and project to the nucleus accumbens (NAc), prefrontal cortex (PFC), amygdala, and hippocampus (Feltenstein & See, 2008; Pierce & Kumaresan, 2006). This pathway is referred to as the mesocorticolimbic pathway and it is implicated not only in SUDs, but also behavioral addictions. Psychostimulant drugs are highly reinforcing due to their ability to increase synaptic monoamine levels. Specifically, cocaine blocks presynaptic reuptake transporters, which results in excess neurotransmitter in the synaptic cleft. Similarly, amphetamines inhibit transport, and they also reverse the transporter not only on the presynaptic terminals but also on the neurotransmitter storage vesicles, causing release of these monoamines. This mechanism appears to be critical for the initial phase of drug use, when the drug produces a pleasurable and positively reinforcing experience. As the drug use becomes chronic, the act of seeking and taking the drug becomes repetitive and habitual. Additionally, the individual learns that stress, anxiety, guilt, and shame can be

temporarily dampened by drug use. This maladaptive learning process and habit formation leads to persistent compulsive behavior to procure drugs, which is a hallmark of dependence in the later stages of SUDs when the negative reinforcing effects (i.e., relief from a negative state) of the drug become prominent. The shift from casual or impulsive use to compulsive drug use suggests that more enduring changes are occurring within this reward circuitry (Kalivas & Volkow, 2005). Such changes involve synaptic plasticity that underlies, at least in part, the enduring changes in behavior (Hyman & Malenka, 2001; Hyman, Malenka, & Nestler, 2006; Kalivas, 2009; Kauer & Malenka, 2007).

Self-administration Animal Model

The drug self-administration paradigm is an operant conditioning model in which an operant response, such as a lever press or nose poke into a hole, is reinforced by the effects of the drug (for review, see Panlilio & Goldberg, 2007). The basic assumption of this model is that the drug or food reward functions as a reinforcer that increases the likelihood of the behavior that preceded its delivery. The relationship between the response and the reinforcement is dictated by the schedule of reinforcement, which sets the number and/or timing of responses required to produce the reinforcer. Additionally, other stimuli, such as a light and/or tone cue, may be presented to signal the availability of reinforcement, i.e., a discriminative stimulus, or may be presented with the delivery of the reinforcer. In the latter case, the cues become associated with the reinforcer through Pavlovian conditioning and may become reinforcing themselves, i.e., a conditioned reinforcer.

Schedules of reinforcement may require a certain number of responses that need

to be performed (fixed-ratio), or a certain amount of time that must pass (fixed-interval), for the reinforcer to be obtained. These requirements may also vary (variable-ratio and variable-interval schedules). For example, a variable ratio schedule 5 (VR5) schedule of reinforcement requires an animal to press a lever on average 5 times before delivery of the reinforcer. Progressive-ratio (PR) schedules assess how effective the reinforcer is by measuring how persistent an animal is in seeking the reinforcer (Arnold & Roberts, 1997). Often the PR schedule of reinforcement is said to assess incentive motivation (Markou et al, 1993). Under the PR schedule, the number of responses required for reinforcement is increased with each successive reinforcer. Thus, it progressively becomes more and more effortful to achieve the reinforcer and eventually the animal ceases performing the operant behavior, or essentially 'gives up'. The highest number of operant responses the animal is willing to perform is termed the breakpoint and is typically defined as the last ratio schedule completed before a "quit" criterion is reached. The breakpoint reflects the amount of 'work' the animal is willing to perform to achieve the reward.

The environment where drug is delivered or stimuli presented with the drug reinforcer, such as the lever, light, and tone are predictive of the drug's availability and/or effects. These stimuli eventually have conditioning effects of their own, similar to the ability of environmental cues associated with drug use in humans to elicit craving and drug-like effects (Childress, McLellan, & O'Brien, 1986; Ehrman, Robbins, Childress, & O'Brien, 1992; Hugdahl & Ternes, 1981; Pomerleau, Fertig, Baker, & Cooney, 1983). Some examples of environmental cues associated with drug use in humans can be the room where the drug is regularly consumed, the paraphernalia used to take the drug, and

the people usually present during the drug-taking experience. This type of conditioning contributes to addiction and relapse.

In the drug self-administration model, the rate of self-injection is dose-dependent and low effort schedules typically produce an inverted U-shaped dose-response function. Infusions rates increase across the low to middle range of doses presumably because the reinforcing effects are increasing as the dose increases, whereas higher doses maintain less frequent responses/injection presumably because the high doses produce a transient satiation effect that results in longer inter-infusion intervals. These low effort schedules are very useful for examining drug reinforcement because the effort required is low, which makes these schedules less reliant on motivation and more reliant on the rewarding properties of the drug than effortful schedules.

Once animals acquire self-administration, there are other manipulations that can be conducted with this model such as extinction/reinstatement and abstinence. These manipulations are typically used to mimic the human relapse condition and to investigate potential pharmacological interventions that can reduce reinstatement of drug-seeking behaviors. During extinction the animal revisits the self-administration chamber everyday but there are no cues or drug available. The animal learns that the operant response no longer produces light/tone cues nor predicts that drug is going to be available. The reinstatement phase begins after either the abstinence or extinction phase. After extinguishing the operant response, animals revisit the self-administration chamber for numerous types of reinstatement tests; cue reinstatement, drug-primed reinstatement, stress reinstatement. Importantly, different pharmacological interventions can be used to test for efficacy in blocking the reinstatement. Stimuli that have previously been

associated with the drug (i.e., paraphernalia, environment, etc.) are conditioned reinforcers. Conditioned reinforcers alone are capable of reinforcing the response that produces them. Also, these conditioned reinforcers can produce conditioned responses that are motivational in nature, leading an individual to seek the drug. These incentive-motivational effects tend to translate to what is being described when humans report craving for a drug.

During forced abstinence, animals remain in their home colony and do not visit the self-administration chambers. Unlike extinction, the association between drug reward and the environment is not extinguished, but rather the animal is removed from the self-administration environment and does not receive any exposure to the drug. During forced abstinence, integrity of the drug-taking behavior and the drug-associated cues conditioned to the drug-taking environment are preserved because the environmental cues associated with the drug are not experienced in the absence period (for review see Reichel & Bevins, 2009). During reinstatement/relapse testing, the drug-reinforced associations such as the lever and the self-administration environmental are fully intact. This model simulates some key aspects of drug relapse in humans. Chronic drug users will be abstinent from the drug for a period of time, whether mandated (i.e., court) or voluntary, before a relapse opportunity presents itself. During this time, there typically is little to no opportunity for drug-taking behaviors or their associated stimuli to be extinguished. Most treatment for SUDs does not include forced extinction of drug-taking behaviors or the stimuli associated with taking the drug (for review, see Ling, Rawson Shoptaw, & Ling, 2006). Cocaine-seeking varies as a function of time since last drug exposure (Tran-Nguyen et al., 1998). Grimm et al., 2001 termed this phenomenon the

"incubation" of craving (Grimm, Hope, Wise, & Shaham, 2001).

Conditioned Place Preference Animal Models

The conditioned place preference (CPP) paradigm is used to study both the rewarding and aversive effects of a drug, food, or other rewarding stimuli. The CPP paradigm involves a classical conditioning process where the animal learns to associate the internal rewarding drug experience (i.e., unconditioned stimulus) with the external environmental stimuli (i.e., conditioned stimulus). The association is later assessed as approach to, and an increase in the amount of time spent in that environment. The model can also be used to assess aversive effects of drugs such as lithium, which are assessed as avoidance of, and a decrease in the amount of time spent in the drug-associated environment (conditioned place aversion; CPA).

The basic CPP procedure involves associating a drug with a particular environment (drug-paired compartment), and on alternating sessions a neutral state becomes associated with an alternate environment (vehicle-paired compartment; for review, see Prus, James, & Rosecrans, 2009). Typically, this is done in a 2-compartment apparatus in which the compartments can differ on 3 modalities; sensory, olfactory, and visual. Some apparatus have a small chamber in between the two main chambers that serves as a gate between the two main compartments. Initially, there are baseline preference tests where the animal has free access to both sides of the apparatus. The animal's least preferred compartment becomes the drug-paired side and the initially preferred compartment becomes the vehicle-paired side. The drug and vehicle sessions alternate, typically resulting in 1-6 exposures to each side. A CPP is established if the animal's preference shifts to the drug-paired compartment during a post-conditioning

preference test.

The CPP model can assess incentive motivational effects of a drug priming injection using an extinction-reinstatement procedure similar to that used in the operant drug self-administration model. After a CPP is established, the extinction phase begins. Animals receive alternating exposures to the drug-paired and vehicle-paired compartments while in a drug-free state to extinguish the association formed between the drug and the drug-paired compartment. Extinction is evident if animals no longer show a preference for the drug-paired compartment when given a preference test. Next, the reinstatement phase assesses incentive motivation produced by a drug priming injection or some other motivation-inducing stimulus such as stress. Manipulations that attenuate reinstatement of the drug CPP are thought to impede neural processes underlying incentive motivational effects of the reinstating stimulus (e.g., drug priming injection).

Sensitization Animal Models

Repeated exposure to psychostimulant drugs can cause a variety of neural and behavioral changes (for review, see Robinson & Berridge, 1993). The most frequently studied of these phenomena is sensitization to effects of these drugs because it is presumed to be an important component of drug addiction (Robinson & Berridge 1993; Robinson & Berridge, 2008; Wolf and Ferrario, 2010). Sensitization is the opposite of tolerance and is sometimes referred to as reverse tolerance. With sensitization, a progressive amplification in behavioral responsiveness occurs after repeated treatment with a psychostimulant drug (Robinson & Becker 1986; Kalivas & Stewart 1991), typically measured as enhanced locomotor activity, rotational behavior or stereotyped motor patterns (Segal, Geyer & Schuckit, 1981; Robinson & Becker, 1986; Robinson &

Berridge, 1993; Stewart & Badiani, 1993). Typically, animals are given a low dose challenge injection of a psychostimulant drug (e.g., cocaine, amphetamine, methylphenidate, methamphetamine, etc.) days or weeks after receiving a sensitizing regimen of repeated daily injections of the drug. Sensitization is evident as an enhanced behavioral response to the challenge either compared to a pre-sensitization test or to a control group receiving the challenge without prior exposure to the stimulant (Browman, Badiani, & Robinson 1998; Kalivas, Duffy, DuMars, & Skinner, 1988; Kuribara & Uchihashi 1994).

Psychostimulant sensitization involves changes in brain mesolimbic dopamine transmission, as well as gene expression within dopamine neurons (for review, see Robinson & Berridge, 2001). An associative process may contribute to behavioral sensitization. Incentive learning occurs when dopaminergic neurons are activated, usually by rewards. Previously neutral stimuli that were associated with the reward acquire incentive salience and are able to elicit incentive motivation that manifests as drug-seeking behavior in animal models (Schmidt & Beninger, 2006). Sensitization is more robust when the animal is re-exposed to the drug in the same environment (context-dependent) as the previous drug exposure, compared to testing in an environment that differs (context-independent) from where drug exposure occurred (Vezina, Giovino, Wise, & Stewart, 1989; Anagnostaras & Robinson, 1996; Wang & Hsiao, 2003; Vezina & Leyton, 2009). Incentive learning is thought to underlie psychostimulant-induced, context-dependent sensitization that plays a prominent role in the development of addiction (Anagnostaras & Robinson, 1996; Pert, Post, & Weiss, 1990). This associative process can explain how environmental stimuli associated with drug taking may increase

craving and increase the risk for relapse in addicts attempting to quit (Robinson & Berridge, 1993). The phenomenon of sensitization can also be due to non-associative learning processes. Neural sensitization without the influence of contextual stimuli has been shown using in-vivo slice electrophysiology (Castaneda, Becker & Robinson, 1988; Robinson & Becker, 1982) and with aplasia (for review, see Kandel & Schwartz, 1982). Therefore, repeated drug exposure can induce sensitization either associatively or non-associatively (i.e., context-independently).

5-HT$_{1B}$Rs and Drug Abuse Circuitry

Historically, mesolimbic dopamine has been highlighted as the mechanism of action responsible for SUDs. However, drugs of abuse, stimulants in particular, modulate not only dopamine neural transmission, but also glutamate, norepinephrine, epinephrine, and serotonin. Serotonin plays a role in the reinforcing and incentive motivational effects of psychomotor stimulants and cues associated with their use (Markou et al., 1993; Shaham, Shalev, Lu, de Wit, Stewart, 2003; for review, see Cruickshank & Dyer, 2009 and Filip, Frankowska, Zaniewska, Gołda, & Przegaliński, 2005). One mechanism involved in these effects is the action of serotonin at 5-HT$_{1B}$ receptors (5-HT$_{1B}$Rs; Clark & Neumaier, 2001; Filip, Alenina, Bader, & Przegaliński, 2010; Neisewander, Cheung, Pentkowski, 2014; Miszkiel, Filip, Przegaliński, 2011). 5-HT$_{1B}$Rs are autoreceptors on 5-HT neuron terminals, including those in the VTA (for review, see Sari 2004), as well as heteroreceptors that modulate GABA and DA release in this region (O'Dell & Parsons, 2004; Yan, Zheng, & Yan, 2004). They are also heteroreceptor on GABAergic striatal neurons that project to the VTA, ventral pallidum, and substantia nigra (Bruinvels et al., 1994; Riad et al., 2000).

Studies investigating the distribution of 5-HT$_{1B}$Rs in the central nervous system have demonstrated that high densities of these receptors are located in the globus pallidus, nucleus accumbens, substantia nigra, and dorsal subiculum (Boulenguez et al., 1992; Bruinvels et al., 1994; Pazos & Palacios 1985; Sari 2004; Sari et al., 1997; Sari et al., 1999). They are also expressed in the striatum and frontal cortex (Barnes & Sharp, 1999; Hoyer, Hannon, & Martin, 2002). 5-HT$_{1B}$Rs are located on the terminals of VTA neurons that project to the NAc, amygdala complex, and frontal cortex (Asan, 1998). The localization of these receptors makes them a prime candidate for regulating the effects of psychostimulants.

5-HT$_{1B}$Rs and Locomotor Activity

5-HT$_{1B}$Rs modulate spontaneous locomotion and cocaine-induced locomotion. Several studies have found that 5-HT$_{1B}$R agonists increase spontaneous locomotor activity in drug-naïve rats (Chaouloff, Courvoisier, Moisan, & Mormede, 1999; Geyer 1996; Koe, Lebel, Fox, & Macor, 1992; Macor et al., 1990; Oberlander, Blaquière, & Pujol 1986; Oberlander, Demassey, Verdu, Van de Velde, & Bardelay, 1987). However, 5-HT$_{1B}$R agonists have no effect on spontaneous locomotion in rats with a history of cocaine self-administration (Pentkowski, Acosta, Browning, Hamilton, & Neisewander, 2009; Przegalinski, Gołda, Frankowska, Zaniewska, & Filip, 2007). The 5-HT$_{1B}$Rs facilitate cocaine-induced hyperlocomotion in both rats and mice (Castanon, Scearce-Levie, Lucas, Rocha, & Hen, 2000; Przegalinski, Filip, Papla, & Siwanowicz, 2001a; Hoplight, Vincow, & Neumaier, 2005). However, in 5-HT$_{1B}$R knockout mice (KO), suppressant effects were found for cocaine-induced hyperlocomotion and the acquisition of sensitization (Rocha et al., 1998). Interestingly, 5-HT$_{1B}$R agonist effects on

spontaneous locomotion may be specific to rats since the drugs have no effect on locomotion in drug-naïve mice (Bannai, Fish, Faccidomo, & Miczek, 2007; Fish, McKenzie-Quirk, Bannai, & Miczek, 2008; Nasehi, Ghadimi, Khakpai, & Zarrindas, 2017). It is important to note this species difference. However, in mice that have been stressed by repeated behavioral testing, the selective 5-HT_{1B}R agonist 5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-pyrrolo[3,2-*b*]pyridine (CP94253) increases locomotion (Tatarczynska, Kłodzińska, Stachowicz, & Chojnacka-Wójcik, 2004; Tatarczyńska, Antkiewicz-Michaluk, Kłodzińska, Stachowicz, & Chojnacka-Wójcik, 2005). Collectively, these findings suggest that 5-HT_{1B}R stimulation enhances locomotion in drug-naïve rats and rats or mice given cocaine or with a history of stress.

5-HT_{1B} receptor stimulation also enhances d-amphetamine-induced locomotor activity and sensitization (Fletcher & Korth, 1999; Przegalinski, Siwanowicz, Nowak, Papla, & Filip, 2001b). 5-HT_{1B} receptor antagonists inhibited d-amphetamine-induced locomotor sensitization (Przegalinski et al., 2001b) or had no significant effect (Chaouloff et al., 1999). The acquisition of amphetamine-induced sensitization is blocked by 5-HT_{1B}R antagonists and facilitated by agonists (Przegalinski et al., 2001b). 5-HT_{1B}Rs in the VTA enhance the amphetamine effects on locomotion when pharmacologically stimulated (Papla, Filip, & Przegalinski, 2002). Surprisingly, 5-HT_{1B}R KO mice are more sensitive to the acute effects of amphetamine and show a more pronounced establishment of amphetamine sensitization (Bronsert, Mead, Hen, & Rocha, 2001). In summary, the majority of research shows a facilitatory role of 5-HT_{1B}Rs in both the acute hyperlocomotor effects of amphetamine and in the establishment of their sensitization.

5-HT_{1B}Rs – Psychostimulant Conditioned Behaviors

Previous research led us to discover that 5-HT_{1B}Rs modulate cocaine-related behaviors in opposite directions depending on whether or not animals have undergone an abstinence period prior to testing (Pentkowski et al., 2012; Pentkowski et al., 2014). More specifically, we observed an increase in cocaine intake with several 5-HT_{1B}R agonists or viral overexpression of 5-HT_{1B}Rs during the maintenance phase of self-administration (Parsons, Weiss, & Koob, 1998; Pentkowski et al., 2012; Pentkowski et al., 2014). In contrast, after a 21-day period of abstinence, either the 5-HT_{1B}R agonist CP94253 or viral over-expression of 5-HT_{1B}Rs attenuates cocaine intake (Pentkowski et al., 2012; Pentkowski et al., 2014). These effects are not due to altering locomotor activity nor reinforcement in general as neither sucrose or food reinforcement is affected (Parsons et al., 1998; Przegalinski et al. 2007; Pentkowski et al., 2009). Furthermore, using a progressive ratio reinforcement schedule, CP94253 has similar paradoxical effects whereby it enhances cocaine intake on the progressive ratio schedule before abstinence, but decreases intake after a period of abstinence (Pentkowski et al., 2014). These findings suggest that 5-HT_{1B}R activation can *increase or decrease* the reinforcing value of cocaine, as well as the motivation for cocaine, depending on whether or not animals have experienced a period of abstinence.

Pharmacological and knockout approaches suggest a facilitatory role for 5-HT_{1B}Rs in the establishment of cocaine CPP in rats and mice (Belzung, Scearce-Levie, Barreau, & Hen, 2000; Cervo et al., 2002). CP94253 potentiates cocaine CPP when a low dose of cocaine is used rats (Cervo et al., 2002). 5-HT_{1B}R KO mice fail to show reliable cocaine CPP, suggesting that stimulation of 5-HT_{1B}Rs is needed to achieve cocaine reward (Belzung et al., 2000). However, cocaine CPP is not affected by

administering a 5-HT_{1B}R antagonist, GR127935, prior to cocaine conditioning sessions in rats (Cervo et al., 2002). This discrepancy may either be due to a species-specific difference in 5-HT_{1B}R modulation of cocaine reward, or to difference between acute pharmacological blockade and the genetic knockout of 5-HT_{1B}Rs which is long-term and may cause changes to compensate for the loss of the receptors. In rats with a virally mediated over-expression of 5-HT_{1B}Rs in projections from the nucleus accumbens to the VTA, there is an increase in cocaine self-administration, suggesting a facilitatory role for VTA 5-HT_{1B}Rs (Pentkowski, 2012). Over-expression of 5-HT_{1B}Rs in the NAc Shell (NAcS) also enhances cocaine-induced CPP (Barot, Ferguson, & Neumaier, 2007).

Given the similarities in pharmacological action of cocaine and methamphetamine, subsequent studies in our lab examined effects of 5-HT_{1B}R agonist administration on methamphetamine self-administration. Surprisingly, methamphetamine intake was attenuated with the administration of CP94253 both before and after abstinence (Garcia, Cotter, Leslie, Olive, & Neisewander, 2017), contrary to our observations with cocaine self-administration. Also, treatment with CP94253 did not alter spontaneous locomotion or inactive lever responses in these rats, thereby indicating that the reduction in lever pressing was not a result of motoric dysfunction (Garcia et al., 2017). Other researchers have shown that 5-HT_{1B}R agonists attenuate d-amphetamine intake without an abstinence phase, as well as responding for a conditioned reward (Fletcher & Korth, 1999; Fletcher, Azampanah, & Korth, 2002; Miszkiel, Adamczyk, Filip, & Przegalinski, 2012; Miszkiel & Przegalinski, 2013). Thus, we suggest that the 5-HT_{1B}R is a good target for developing therapeutics for cocaine and methamphetamine addiction due to its 1) location in the reward circuitry, 2) ability to modulate release of

multiple neurotransmitter systems, and 3) potential regulatory role in drug-seeking behaviors.

Fos Protein Expression

C-fos is a proto-oncongene that is expressed in neurons following depolarization. It belongs to a family of early immediate gene (EIG) transcription factors that are activated by a broad range of extracellular stimuli and are induced rapidly and transiently. Due to these characteristics of the c-fos gene, its induction is considered an indicator of brain changes in gene expression. Indeed, transcription factors are proteins that control the rate of transcription of genetic information from DNA to messenger RNA. *C-fos* is induced by a variety of stimuli relevant to signal transduction within the nervous system, including growth factors (Fisch, Prywes, & Roeder, 1987; Rivera & Greenberg 1990), depolarization (Sheng, Dougan, McFadden, & Greenberg, 1988; Sheng, McFadden, & Greenberg, 1990; McFadden, & Greenberg, 1990), calcium entry (Fisch et al. 1987; Sheng et al., 1988), the cyclic AMP/protein kinase A pathway (Sassone-Corsi, Visvander, Ferland, Mellon, & Verma, 1988; Fisch, Prywes, Simon, & Roeder, 1989), the protein kinase C pathway (Fisch et al. 1987; Gilman 1988), and others. Because many IEGs are induced in the nervous system in response to neurotransmitters and other physiological stimuli, these genes may play an important role in the function of the nervous system (for review, see Sheng & Greenberg, 1990). Since *C-fos* is activated in response to neuronal activity, immunohistochemical detection of the *C-fos* protein product, Fos, is a widely used tool to map activation of cells in the nervous system in response to many stimuli, including drugs.

The family of fos proteins (i.e., *C-fos*, fos-b, jun proteins) has been implicated in

regulation of cell proliferation and differentiation. Drugs of abuse have been shown to alter many types of transcription factors in a variety of brain regions (O'Donovan, Tourtellotte, Millbrandt, & Baraban, 1999; Berke & Hyman, 2000; Nestler, Hyman, & Malenkam 2001a; Mackler, Homan, Korutla, Conti, & Blendy, 2003). Administration of psychostimulants and opioid drugs causes the rapid and transient induction of several *fos* and *jun* proteins in the NAc and caudate-putamen (CPu; Graybiel, Moratalla, & Robertson, 1990; Nestler, Barrot, & Self, 2001b; Young, Porrino, & Iadarola, 1991; for a review, see Harlan & Garcia, 1998). Fos protein expression is also altered in the PFC and VTA in response to cocaine and other psychostimulants (Fanous, Lacagnina, Nikulina, & Hammer, 2011; Kufahl et al., 2009; Mahler & Aston-Jones, 2012). Not only can IEGs serve as indicators of neuronal activity, but they are also thought to represent an important initial step in mediating drug experience-dependent plasticity (Hyman & Malenka, 2001; Nestler, 2001c). The NAc, together with dopaminergic neurons in the VTA that innervate the NAc, mediate psychological aspects of addiction, namely, drug reinforcement and craving, for opiates and many other drugs of abuse (Wise & Bozarth 1987; Koob & Bloom, 1988; Clouet, Asghar, & Brown, 1988). Therefore, we can use Fos expression as a reliable marker to identify the brain regions involved in various aspects of addiction. In particular, we can examine brain region specificity in response to the drug itself, drug-related cues, abstinence from the drug, or reinstatement of drug-seeking behavior.

Aims of this Dissertation Research

This dissertation aimed to examine the role 5-HT_{1B}Rs play in regulating cocaine and methamphetamine abuse-related behavior in mice. Much of the research

investigating the pre- and post-abstinence effects of 5-HT_{1B}R agonists has been conducted in rats. We were specifically interested in the 5-HT_{1B}R agonist CP94253 in cocaine and methamphetamine induced behaviors. I initially hypothesized that CP94252 would have a faciliatory effect on cocaine-induced locomotion prior to abstinence but would block cocaine-induced sensitization after abstinence, similar to the opposing effects of the drug on cocaine self-administration observed by our lab in rats. I tested this hypothesis by examining the effects of CP45953 on cocaine-induced locomotor activity after a daily cocaine injection regimen for 21 days in mice who were subsequently test after 1 and 21 days of abstinence after the last injection. Next, I investigated the effect of CP94253 on the reinstatement of cocaine-primed CPP in mice. I hypothesized that mice initially exhibiting a CPP and having undergone extinction would reinstate their CPP when given a cocaine-prime injection prior to the reinstatement test, and that CP94253 would block that reinstatement. I then examined the effects of CP94253 both on the acquisition and expression of methamphetamine-CPP. I hypothesized that CP94253 would block both the acquisition of methamphetamine-CPP and the expression of methamphetamine-CPP. However, only the latter effect was observed. Lastly, I examined the potential circuitry underlying the CP94253-induced attenuation of the expression of methamphetamine-CPP by examining 1) Fos protein expression in regions of the mesocorticolimbic pathways, and 2) the types of neurons affected by the CP94253 by co-labelling for Fos with glutamic acid decarboxylase (GAD) in GABA neurons and tyrosine hydroxylase (TH) on DA neurons. I accomplished this by harvesting the brain after the methamphetamine expression test. Based on previous research, I predicted that mice expressing methamphetamine-CPP would have increased levels of Fos expression

in the NAcS, NAc Core (NAcC), VTA, basolateral amygdala (BLA), and prelimbic cortex (PrL). Lastly, as a control procedure I investigated the effect of acute CP94253 and methamphetamine on unconditioned Fos in the same brain regions.

CHAPTER 2

EFFECTS OF A 5-HT$_{1B}$ RECEPTOR AGONIST ON LOCOMOTION AND REINSTATEMENT OF COCAINE-CONDITIONED PLACE PREFERENCE AFTER ABSTINENCE FROM REPEATED INJECTIONS IN MICE

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Abstract

5-HT$_{1B}$ receptors (5-HT$_{1B}$Rs) modulate behavioral effects of cocaine. Here we examined the effects of the 5-HT$_{1B}$R agonist CP94253 on spontaneous and cocaine-induced locomotion and on cocaine-primed reinstatement of conditioned place preference (CPP) in male mice given daily repeated injections of either saline or cocaine (15 mg/kg, IP) for 20 days. In the locomotor activity experiment, testing occurred both 1 and 20 days after the final injection. In the CPP experiment, mice underwent conditioning procedures while receiving the last of their daily injections, which were given either during or ≥2 h after CPP procedures. The CPP procedural timeline consisted of baseline preference testing (days 12-13 of the chronic regimen), conditioning (days 14-19, 2 daily 30-min sessions separated by 5 h), CPP test (day 21), extinction (days 22-34; no injections), CPP extinction test (day 35), and reinstatement test (day 36). Mice that had not extinguished received additional extinction sessions prior to reinstatement testing on day 42. On test days, mice were pretreated with either saline or CP94253 (10 mg/kg, IP). Testing began 30 min later, immediately after mice were primed with either saline or cocaine (5 mg/kg for locomotion; 15 mg/kg for reinstatement). We found that CP94253 increased spontaneous locomotion in mice receiving repeated injections of either saline or cocaine when tested 1 day after the last injection, but had no effect on spontaneous locomotion

after 20 days abstinence from repeated injections. Surprisingly, cocaine-induced locomotion was sensitized regardless of whether the mice had received repeated saline or cocaine. CP94253 attenuated expression of the sensitized locomotion after 20 days abstinence. A control experiment in noninjected, drug-naïve mice showed that CP94253 had no effect on spontaneous or cocaine-induced locomotion. Mice reinstated cocaine-CPP when given a cocaine prime, and CP94253 pretreatment attenuated the cocaine reinstatement. The findings suggest that stress from repeated saline injections and/or co-housing with cocaine-injected mice may cross-sensitize with cocaine effects on locomotion and that CP94253 attenuates these effects, as well as reinstatement of cocaine-CPP. This study supports the idea that 5-HT_{1B}R agonists may be useful anti-cocaine medications.

Introduction

Serotonin plays a role in the reinforcing and incentive motivational effects of cocaine and cocaine-associated cues (Markou et al., 1993; Shaham et al., 2003). One mechanism involved in these effects is the action of serotonin at 5-HT_{1B} receptors (5-HT_{1B}Rs; Clark & Neumaier, 2001; Filip et al., 2010; Neisewander et al., 2014; Miszkiel et al., 2011). Parsons and colleagues discovered that 5-HT_{1B}R agonists shift the cocaine self-administration (SA) dose-effect function to the left and increase responding on a PR schedule of cocaine reinforcement, suggesting enhanced reinforcing value of cocaine (Parsons et al., 1998). These 5-HT_{1B}R agonist effects are reversed by a 5-HT_{1B}R antagonist, demonstrating that they are 5-HT_{1B}R-mediated. Furthermore, the agonists do not alter sucrose or food reinforcement or locomotion at doses that enhance the reinforcing value of cocaine (Parsons et al., 1998; Przegalinski et al. 2007; Pentkowski et

al., 2009). Surprisingly, we found that both cue and cocaine-primed reinstatement of cocaine-seeking behaviors are attenuated by 5-HT_{1B}R agonists (Acosta et al., 2005; Pentkowski et al., 2009). These seemingly paradoxical findings led us to discover that 5-HT_{1B}Rs modulate cocaine-related behaviors in opposite directions depending on whether or not animals have undergone an abstinence period prior to testing (Pentkowski et al., 2014). Specifically, either the agonist 5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-pyrrolo[3,2-*b*]pyridine (CP94253) or viral overexpression of 5-HT_{1B}Rs tested during the maintenance of daily self-administration sessions *increased* the reinforcing value of cocaine, measured as a leftward shift of the cocaine self-administration dose-effect function on low ratio schedules of reinforcement and an increase in intake on a progressive ratio schedule (Pentkowski et al., 2012, 2014). In contrast, after a 21-day period of protracted abstinence, the agonist *attenuated* cocaine intake at the same low dose of cocaine (0.075 mg/kg, IV) for which CP94253 had enhanced intake prior to an abstinence period (Pentkowski et al., 2014) and attenuated intake on a progressive ratio schedule of cocaine reinforcement. These findings demonstrate opposite functional effects of 5-HT_{1B}R agonists pre- versus post-abstinence from cocaine self-administration.

5-HT_{1B}Rs also modulate spontaneous locomotion and cocaine-induced locomotion under some circumstances. Several studies have found that 5-HT_{1B}R agonists stimulate locomotor activity in drug-naïve rats (Chaouloff et al., 1999; Geyer 1996; Koe et al., 1992; Macor et al., 1990; Oberlander et al., 1986; Oberlander et al., 1987), but have no effect on spontaneous locomotion in rats with a history of cocaine self-administration (Pentkowski et al., 2009; Przegalinski 2007). 5-HT_{1B}R agonist effects on spontaneous locomotion may be specific to rats since the drugs have no effect in drug-naïve mice

(Bannai et al., 2007; Fish et al., 2008; Nasehi et al., 2017). However, in mice that had been stressed by repeated behavior testing, CP94253 increases locomotion (Tatarczynska et al., 2004; Tatarczynska et al., 2005). Additionally, the 5-$HT_{1A/1B}$R agonist RU24969 dose-dependently increases spontaneous locomotion in wild type mice, but not 5-HT_{1B}R KO mice (Saudou et al., 1994). CP94253, as well as another 5-HT_{1B}R agonist CP93129, have been shown to potentiate cocaine-induced locomotion and cocaine sensitization in rats (Filip et al., 2010; Przegalinski et al., 2001a; Przegaliński, Siwanowicz, Papla, & Filip, 2002; Przegalinski, Papla, Siwanowicz, & Filip, 2004). Collectively, these findings suggest that 5-HT_{1B}R stimulation enhances locomotion in rodents given cocaine or with a history of stress.

One goal of the present study was to examine whether the abstinence-induced "switch" in 5-HT_{1B}R functional modulation of cocaine-related behaviors observed in rats previously is also observed in mice. To this end, we investigated whether CP94253 produces opposing effects on spontaneous and cocaine-induced locomotion before and after an abstinence period in C57BL/6 male mice receiving daily injections of either saline or cocaine (15 mg/kg, IP) for 20 days. The second goal was to investigate whether the incentive motivational effects of a cocaine priming injection are attenuated by 5-HT_{1B}R agonist treatment in mice that had undergone abstinence, similar to the decrease in cocaine-primed reinstatement of cocaine-seeking behavior observed previously in rats (Pentkowski et al., 2012; Pentkowski et al., 2014). To this end, we investigated CP94253 effects on cocaine-primed reinstatement of extinguished cocaine-conditioned place preference (CPP).

Methods

Animals

Male C57BL/6 mice arrived at 5 weeks old from Jackson Laboratories (Sacramento, CA) and were group housed 3-4/cage in a climate-controlled facility with a reversed 10 h light/14 h dark cycle (lights off at 6:00 AM). Mice were handled for 2 weeks. For the CPP experiment only, mice were transferred to single housing 1 day prior to the start of behavior testing. Food and water were provided *ad libitum* in the home cage. All behavioral testing occurred between 8 AM and 4 PM. Separate groups of experimentally naïve mice were used for each specific experiment. All husbandry and procedures adhered to the Guide for the Care and Use of Laboratory Animals (2011), and all experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Arizona State University.

Drugs

Cocaine hydrochloride (RTI International, Research Triangle Park, NC) and CP94253 (Tocris Bioscience, Minneapolis, MN) were dissolved in bacteriostatic saline. All drugs were injected at a volume of 1 ml/100 mg of body weight. The doses used had been previously reported to produce cocaine- (Rao, Sorkin, A., & Zahniser, 2013; Shuman, Cai, Sage, & Anagnostaras, 2012; Tilley et al., 2007) and CP94253-induced hyperlocomotion in mice injected 30 min before testing (Bannai et al., 2007; Fish et al., 2008; Tatarczynska et al., 2004; Tatarczynska et al., 2005).

Apparatus

Locomotor activity tests were conducted in Plexiglas chambers, each measuring 35×24×31 cm high. The chambers had corn cob bedding on an acrylic floor and alternating black and white stripes on the walls. CPP experiments were conducted in

Plexiglas two-compartment apparatus with each end compartment measuring 35×24×31 cm high and with a removable partition separating them. One compartment had cedar bedding beneath a wire 1×1 cm grid floor and alternating black and white vertical stripes on the walls. The other compartment had pine bedding beneath a parallel bar floor (5 mm diameter) and alternating black and white horizontal stripes on the walls. In order to prevent the mice from escaping from the chambers while maintaining the ability to record their behavior via an overhanging video camera, a rectangular tower measuring 70×24×74 cm high of clear Plexiglas was used as an extension of the apparatus. The testing room was dimly lit with two overhead lamps, each containing a 25 Watt light bulb. A camera (Panasonic WV-CP284, color CCTV, Suzhou, China) used to record testing sessions was mounted 101 cm above the center of each apparatus. A WinTV 350 personal video recorder (Hauppage, NJ, USA) captured live video encoded into MPEG streams. A modified version of TopScan Software (Clever Sys., Inc. Reston, VA, USA) was used to track the animals' movement. This program uses the orientation of an animal's body parts (e.g. nose, head, center of body, forepaws, base of tail, etc.) to identify the animal's location and specified behaviors.

Experiment 1: Effects of CP94253 on spontaneous and cocaine-induced locomotion before and after chronic daily injections of cocaine or saline

The timeline for Experiment 1 is shown in Fig. 1A. Adult, male C57BL/6 mice (n=91) were housed 4/cage, with 2 mice in each cage assigned to receive saline and 2 assigned to receive cocaine (15 mg/kg, IP) at the same time of day for 20 consecutive days. The mice were further assigned to receive two different pretreatments on the test days. The first pretreatment was either vehicle or CP94253 (10 mg/kg, IP) and the second

pretreatment was either a saline or cocaine (5 mg/kg, IP) challenge injection. Thus the design of this experiment was a 2 (chronic saline or cocaine) X 2 (vehicle or CP94253 pretreatment) X 2 (saline or cocaine challenge) factorial with 8 treatment groups (n=8-11/group). Test day 1 took place on the day after the last chronic injection. After test 1, the mice underwent a 20-day period of no injections during which they remained in the colony room and their tails were marked twice per week to maintain identification. Test day 2 took place the day after the final abstinence (i.e., no injection) day. On both of the test days, mice were first placed into the test chamber for 1 h to allow for habituation. Immediately following this baseline period, mice were injected with either vehicle or CP94253 and were returned to their home cage for 30 min. Next, mice received the saline or cocaine challenge injection and were returned to the test chamber for an additional 60 min. We used a lower cocaine dose for the challenge (5 mg/kg) on test day than that used during the daily repeated administration (15 mg/kg). This was done in order to avoid potential ceiling effects for detecting sensitization of locomotion, a well-known effect of repeated cocaine administration (Ago, Nakamura, Baba, & Matsuda, 2008; DiRocco, Scheiner, Sindreu, Chan, & Storm, 2009; Luo et al., 2010; Riday, Kosofsky, & Malanga, 2012; Robison et al., 2013; Thompson, Martini, & Whistler, 2010).

Experiment 2: Effects of CP94253 on spontaneous and cocaine-induced locomotion in mice without the repeated injection regimen

In order to assess potential injection stress effects, we repeated Experiment 1 using identical procedures and timeline except that the 5 week old, male C57BL/6 mice (n=47) did not receive any injections during the first 20 days of the experiment. Thus, the 4 mice/cage were simply handled twice a week to color-mark tails for identification

purposes and were otherwise left undisturbed to minimize stress. The design was a 2 (vehicle or CP94253 pretreatment) X 2 (saline or cocaine challenge) factorial with 4 treatment groups (n=11-12/group). Test day procedures were identical to Experiment 1.

Experiment 3: Effects of CP94253 on reinstatement of extinguished cocaine-CPP

The timeline for Experiment 3 is shown in Fig. 1B. Adult, male C57BL/6 mice received daily injections of cocaine (15 mg/kg, IP) or saline for 11 days in order to keep the same number of cocaine injections prior to testing for effects of CP94253 in this experiment as that given in the previous experiments. Also, the mice were housed 3/cage and all 3 mice/cage were assigned to the same chronic drug condition. On day 12 and 13 the mice were allowed free access to both sides of the CPP apparatus for 15 min to habituate them to the novel environments and to assess initial compartment preference. The average of the time spent in the least preferred compartment on days 12 and 13 was used as the baseline preference measure. On both days 12 and 13, mice received their chronic daily injection (saline or cocaine) in their home cage 2-3 hours after the preference test. On days 14-19, the mice underwent 2 daily 30-min conditioning sessions separated by a 5-hour period. During the morning session, mice were injected with saline and were placed into their initially preferred side and during the afternoon session mice were injected with cocaine (15 mg/kg, IP) or saline and were placed into their initially non-preferred side. On day 20, mice were not exposed to the apparatus, but did receive either saline or cocaine (15 mg/kg, IP) at the same time of day as all previous injections. On day 21, mice were tested for the expression of cocaine CPP for 15 min. Only 80% of the mice met the CPP expression criterion (spent >450 seconds in initially non-preferred compartment) and continued in the experiment. These mice next underwent extinction

training on Days 22-34. During extinction, the mice received one 30-min exposure to one of the compartments each day, with the particular compartment alternating across the days. On day 35, mice were tested for 15 min to demonstrate that their CPP had extinguished. Mice that extinguished were tested for reinstatement of CPP the following day (day 36). On test day, mice received either saline or CP94253 (10 mg/kg, IP) 30 min prior to the test. Immediately before the test, the mice were primed with either saline or cocaine (15 mg/kg, IP). Mice that did not initially extinguish received 4 more days of extinction with 2, 30-min sessions per day, one in each compartment. They again received a 15-min preference test to demonstrate that their CPP had extinguished. Mice that extinguished were tested for reinstatement of CPP the following day. Mice that failed to extinguish were removed from the study. The design of the study was a 2 (vehicle or CP94253 pretreatment) X 2 (saline or cocaine challenge) factorial with 4 treatment groups (n=9-11/group). Additionally, a group of mice (n=14) were treated chronically with saline, conditioned with saline during both daily sessions, extinction-trained, and given a saline prime prior to testing (i.e., saline control group).

Statistics

Drug-induced changes in distance travelled (meters) were analyzed and graphed for the first 30 min of each testing session. Analyzing only 30 min of the testing session was done because cocaine is rapidly metabolized in mice (Rao et al., 2013; Tilley et al., 2007) and the difference from baseline calculation controlled for individual differences in baseline activity. The change in distance traveled measures were analyzed by mixed factor ANOVAs with the following between group variables: Chronic treatment with cocaine or saline (Experiment 1 only); Pretreatment with CP92453 or vehicle; Challenge

with cocaine or saline prior to test. The ANOVAs also included Test day as a within subjects repeated measure. Interactions were further analyzed by smaller ANOVAs and t-test with Bonferroni correction for multiple comparisons where appropriate. In addition, planned comparisons were conducted to test our hypothesis that CP94253 would enhance spontaneous locomotion and cocaine-induced locomotion pre-abstinence, but would have the opposite effect post-abstinence. Mice whose distance travelled score was more than ±2 standard deviation from the mean were deemed outliers and removed from all analysis. For CPP, time spent in the initially non-preferred side was analyzed by ANOVA with test days as a repeated measures. The test days included the baseline preference test, the CPP test (occurred after six daily pairings with cocaine), and the extinction test (occurred after 18-22 sessions of extinction). This analysis was a manipulation check to demonstrate that cocaine-conditioned rats exhibited CPP and extinction of CPP. To analyze cocaine-primed reinstatement of CPP, time spent in the initially non-preferred compartment of the apparatus (drug-paired compartment) was analyzed by a 2 (Pretreatment: CP94253 and vehicle) X 2 (Priming injection: Cocaine and saline) AVOVA. Interactions were analyzed by smaller ANOVAs and Tukey post-hoc tests.

Results

Experiment 1: Effects of CP94253 on spontaneous and cocaine-induced locomotion before and after chronic daily injections of cocaine or saline

We first tested the hypothesis that mice given chronic cocaine treatment would exhibit a "switch" in 5-HT_{1B}R agonist effects from facilitation of cocaine-induced locomotion during the treatment phase to inhibition of cocaine-induced locomotion after a period of abstinence from chronic cocaine. Surprisingly, the chronic saline group

behaved similarly to the chronic cocaine group (Fig. 2, panels A and B) and the analysis confirmed that there was no main effect nor interactions with chronic treatment (i.e., chronic saline vs. cocaine). Therefore, subsequent analyses were conducted with the data are averaged across chronic condition as shown in Fig. 3A. This analysis revealed a main effect of Challenge, where the cocaine challenge increased locomotion compared to the saline challenge when averaged across pretreatment with Vehicle or CP94253 [$F(1,87)=62.28$, $p<0.001$]. However, there was also a Challenge by Day interaction [$F(1,87)=15.47$, $p<0.001$] as shown in Fig. 3B. Subsequent pairwise comparisons with Bonferroni correction indicated that cocaine-challenged mice showed no difference in locomotion across test days, whereas saline challenged mice showed a decrease in locomotion after abstinence compared to before abstinence [$t(43)=5.8$, $p<0.001$]. There was also a Pretreatment by Day interaction [$F(1,87)=32.83$, $p<0.001$] as shown in Fig. 3C. Subsequent pairwise comparisons indicated that mice pretreated with vehicle showed no difference in locomotion across test days, whereas mice pretreated with CP94253 showed less locomotion after abstinence compared to before abstinence [Bonferroni t-test, $t(44)=5.8$, $p<0.001$]. In addition to the ANOVAs, planned comparisons were conducted to test the hypothesis that CP94253 pretreatment would facilitate spontaneous and cocaine-induced locomotion before abstinence but inhibit these behaviors after abstinence. The results of these comparisons indicated that there was a significant increase in spontaneous locomotion after the CP94253 pretreatment compared to vehicle pretreatment in mice challenged with saline before abstinence from repeated injections [$t(42)=3.0$, $p<0.01$, Fig. 3A]. In mice challenged with cocaine, there was no difference in cocaine-induced locomotion between vehicle- and CP94253-pretreated mice before

abstinence, but the CP94253-pretreated mice showed less cocaine-induced locomotion than vehicle-pretreated mice after abstinence [t(45)=3.6, $p<0.05$, Fig. 3A].

Experiment 2: CP94253 has no effect in mice that have not undergone a repeated injection regimen

The finding that chronic cocaine versus chronic saline treatment did not show differences in locomotion in the previous experiment was puzzling. We reasoned that stress experienced by the saline control group may have cross-sensitized the mice to cocaine such that both groups (i.e., chronic cocaine and chronic saline) showed sensitized responses to cocaine (Sorg 1992). Indeed, the control mice experienced repeated injections and were housed with cocaine-treated mice, and both of these manipulations are chronic stressors in mice (Hoplight, Vincow, & Neumaier, 2007; Ryabinin, Wang, & Finn, 1999). Another concern was that rather than CP94253 having opposite effects on cocaine-induced locomotion before and after abstinence from repeated injections, perhaps the agonist simply has opposite effects the first time it is given compared to the second time it is given. We examined these possibilities in this experiment. Naïve, non-injected mice arrived at the same age as in the previous experiment and were housed for 20 days during which they were handled twice weekly to color-mark tails for identification purposes and were otherwise left undisturbed. As expected, cocaine increased locomotion to a similar degree on the first (day 21) and second (day 42) test days as there was a main effect of Challenge [F(1,43)=15.15, $p<0.001$], but no interactions with Pretreatment or Day. In contrast to the effects of CP94253 observed in the repeatedly injected saline controls (Fig. 2A), CP94253 had no effects on locomotion in injection-naive mice (Fig. 4). This finding suggests that the saline injections in mice from the previous experiment

did indeed produce stress that affected spontaneous and cocaine-induced locomotor activity in a 5-HT_{1B}R-sensitive manner.

Experiment 3: CP94253 prevents cocaine-primed reinstatement of extinguished cocaine CPP

Approximately 40% of the mice preferred the side of the apparatus with horizontal stripes and ~60% preferred the side with vertical stripes, confirming the use of an unbiased apparatus. A repeated measures analysis across the baseline, CPP, and extinction tests showed a significant day by conditioning treatment interaction [$F(2, 106)=13.23$, $p<0.001$; Fig. 5A]. Subsequent analyses comparing saline to cocaine conditioned groups on each test day showed a group difference on the CPP test day but no difference during baseline or extinction [Bonferroni t-test $t(51)=3.98$, $p<0.001$]. These results indicate that cocaine conditioning produced CPP that was abolished by extinction training. In the cocaine conditioned groups, a 2 X 2 ANOVA of time spent in the drug-paired side during the reinstatement test revealed a significant Pre-treatment X Priming injection interaction [$F(1,35)=4.26$, $p<0.05$; Fig. 5B]. Subsequent post hoc analyses indicated that the cocaine-primed, saline-pretreated group showed significantly greater CPP than all other groups (Tukey tests, $p<0.05$). In addition comparisons of each group to its extinction baseline indicated that only the cocaine-primed group showed a significant increase in time spent in the drug-paired side relative to extinction baseline [$t(10)=4.1$, $p<0.005$]. Finally, the cocaine-primed, saline-pretreated group also showed a significantly greater amount of time spent in the drug-paired side relative to the saline controls [$t(23)=2.4$, $p<0.05$]. These results suggest that CP94253 attenuated cocaine-primed reinstatement of cocaine CPP.

Discussion

This study yielded partial support for our hypothesis that mice would show a similar abstinence-dependent change in 5-HT_{1B}R modulation of cocaine effects as observed previously in rats (Pentkowski et al., 2009; Pentkowski et al., 2012; Pentkowski et al., 2014). We predicted that the 5-HT_{1B}R agonist CP94253 would facilitate cocaine-induced locomotion in mice given repeated daily injections of cocaine, but would inhibit this behavior after a 20-day period of abstinence, similar to the "switch" in 5-HT_{1B}R agonist effects observed in rats before and after abstinence from cocaine self-administration. Surprisingly, we found that CP94253 effects on locomotion were the same regardless of whether or not the mice received repeated injections of saline or cocaine (Fig. 2A and 2B). We then conducted further analyses without the chronic treatment as a factor (Fig. 3A). We found that acute administration of CP94253 initially increased spontaneous locomotion in mice tested on the 21st day of their chronic injections as predicted; however, the agonist did not alter spontaneous locomotion after a 21-day abstinence phase. Also, the effects of the agonist on cocaine-induced locomotion only partially supported our predictions because CP94253 failed to alter this behavior initially, but did reverse the cocaine-sensitized hyperlocomotion observed after 20 days abstinence from daily repeated injections. Overall, the results are generally consistent with previous findings in rats of a facilitatory effect on cocaine-induced behavior prior to abstinence and an inhibitory effect after a prolonged period of abstinence.

We had expected that the chronic repeated cocaine injections would sensitize mice to the cocaine challenge given on the first test day and that this effect would be evident as greater locomotor activity in the chronic cocaine-injected group relative to the

chronic saline-injected control group. Because there was no difference between these groups, we speculated that our chronic repeated saline injections may have stressed the mice in the experiment resulting in stress-induced cross-sensitization. Previous research has demonstrated cross-sensitization between repeated stress and repeated cocaine injections in both rats and mice (Boyson et al., 2014; Kikusui, Faccidomo, & Miczek, 2005; Maeda et al, 2006; Prasad, Sorg, Ulibarri, & Kalivas, 1995; Sorg 1992), and repeated injections are stressful in both mice and rats (Ferguson, Sandygren, & Neumaier, 2009; Ryabinin et al., 1999). Another possible stressor was that the control mice were cohoused with the cocaine-treated mice, which may have resulted in chronic social stress. Although we did not notice overt signs of stress such as aggression, Hoplight and colleagues (2007) have previously shown that saline-injected rats pair housed with cocaine-injected rats have altered $5HT_{1B}R$ profiles similar to that of cocaine treated rats, but not those housed with saline treated rats. To test this stress cross-sensitization hypothesis, we examined spontaneous and cocaine-induced locomotion in mice that were group housed and left undisturbed for 20 days except for tail-marking twice/week. In these control mice, the second cocaine challenge failed to sensitize locomotion in contrast to the sensitized locomotion observed in mice were co-housed with cocaine-injected mice and given chronic saline injections. Furthermore, CP94253 failed to alter either spontaneous or cocaine-induced locomotion on either test day in the noninjected control mice. It is important to note that these control mice were tested on two separate occasions after receiving CP94253 pretreatment, mitigating the idea that CP94253 may simply produce different effects the first versus second time it is given. The different pattern of behavior across the chronic saline-injected and noninjected mice,

coupled with the similar pattern of behavior in the chronic cocaine-injected and chronic saline-injected mice, support the interpretation that stress from repeated injection and living with cocaine-injected mice cross-sensitized the mice to cocaine. CP94253 reversed expression of the sensitized locomotion after a period of abstinence. Although the neural mechanism underlying the stress cross-sensitization effects will require further investigation, one likely pathway contributing to these effects is the 5-HT1BR-expressing medium spiny neurons projecting from nucleus accumbens (NAc) shell to the VTA. Previous research has shown that 5-HT_{1B}R located on GABAergic projection neurons from the nucleus accumbens (NAc) shell to the VTA may mediate stress cross-sensitization with psychostimulant drugs (Furay, McDevitt, Klaus, Miczek, & Neumaier, 2011; Nair, Furay, Liu, & Neumaier, 2013; Miczek, Nikulina, Shimamoto, & Covington, 2011).

Although we had predicted that CP94253 would attenuate cocaine-sensitized locomotion after a period of abstinence, a previous study by Przegalinski and colleagues (2001b) showed that while CP94253 dose-dependently enhances hyperlocomotion produced by acute amphetamine administration in mice, it does not affect amphetamine sensitization. The present findings seem discrepant with those of Przegalinski and colleagues (2001b) however, we suggest that CP94253 may differentially alter locomotion induced by cocaine versus amphetamines based on recent work from our laboratory demonstrating a different pattern of changes in cocaine versus methamphetamine self-administration. Unlike the enhancement of cocaine self-administration prior to abstinence (Pentkowski et al., 2009; Pentkowski et al., 2012), CP94253 reduces methamphetamine self-administration both before and after abstinence

(Garcia et al., 2017).

As we had predicted, CP94253 attenuated the cocaine-primed reinstatement of extinguished cocaine-CPP in mice that had a history of chronic cocaine administration followed by protracted abstinence prior to testing. Neither CP94253 pretreatment alone nor a saline prime prior to reinstatement testing altered preference. These control data suggest that reinstatement was specific to cocaine priming and that CP94253 specifically reversed the cocaine priming effect rather than nonspecifically altering preference. The findings are consistent with previous research suggesting that 5-HT_{1B}R agonists attenuate incentive motivational effects of cocaine priming injections in the operant extinction/reinstatement model (Pentkowski et al., 2014; Przegalinski et al., 2002; Przegalinski et al., 2007). Collectively, the studies suggest that 5-HT_{1B}Rs modulate the incentive motivational effects of a cocaine prime in both rats and mice (Fletcher et al., 2002; Parsons et al,. 1998; Pentkowski et al., 2012; Pentkowski et al., 2014).

Demonstrating effects of 5-HT_{1B}R agonists on psychostimulant-induced and conditioned behaviors in mice is important because transgenic mice are a valuable tool for investigating the neural mechanisms of these behaviors. A leading hypothesis for the effects of the agonists on cocaine-induced behaviors suggests that 5-HT_{1B}Rs inhibit either GABAergic interneurons in the VTA or GABAergic medium spiny neurons projecting from the NAc to VTA, and this action disinhibits DA neurons (Barot et al., 2007; Hoplight et al., 2007; Neumaier et al., 2002; O'Dell &Parsons, 2004; Parsons, Koob, & Weiss, 1999; Yan & Yan, 2001). For instance, a microdialysis study suggests that stimulating 5-HT_{1B}Rs in the VTA inhibits GABA release from the neurons that tonically inhibit mesolimbic DA neurons. This leads to disinhibition of the mesolimbic DA

neurons, increasing dopaminergic transmission in the NAc (O'Dell & Parsons, 2004). Because viral-mediated overexpression of 5-HT_{1B}Rs in this pathway attenuates cocaine intake after abstinence (Pentkowski et al., 2012), it is likely that cocaine abstinence causes adaptations within the 5-HT_{1B}R→GABAR→DA circuit in the VTA, which may underlie the inhibitory effects of 5-HT_{1B}R agonists on cocaine-induced behaviors that are observed following protracted abstinence. Transgenic mice may be useful in elucidating the neural circuitry involved in 5-HT_{1B}R agonists effects on cocaine-induced behavior.

In conclusion, this study demonstrates that a 5-HT_{1B}R agonist reverses expression of cocaine sensitization and blocks cocaine-primed reinstatement of cocaine-CPP in mice. These findings offer further support for the idea that serotonin inhibits incentive motivational effects of cocaine through an action at 5-HT_{1B}Rs. Furthermore, this research suggests that 5-HT_{1B}Rs may be a useful target for developing medications for cocaine use disorders and that mice are a useful model for screening the potential anti-cocaine therapeutic effects of 5-HT_{1B}R agonists, as well as for investigating the neural mechanisms involved 5-HT_{1B}R-mediated inhibition of the incentive motivational effects of cocaine.

CHAPTER 3

5-HT$_{1B}$ RECEPTOR AGONIST ATTENUATES EXPRESSION OF METHAMPHETAMINE-CONDITIONED PLACE PREFERENCE AND REVERSES FOS EXPRESSION CHANGES IN MALE MICE

Abstract

We investigated whether 5-HT$_{1B}$ receptors (5-HT$_{1B}$Rs) modulate methamphetamine (METH) reward and/or incentive motivation by measuring the effect of the 5-HT$_{1B}$R agonist CP94253 on the acquisition and expression, respectively, of methamphetamine conditioned place preference (CPP) in C57BL/6 male mice. In the acquisition experiment, mice were pretreated with CP94253 (10 mg/kg, IP) 30 min before receiving methamphetamine (3 mg/kg, IP) during the conditioning procedure. For the expression experiment, rats that had acquired methamphetamine-CPP were given either saline or CP94253 (10 mg/kg, IP) 30 min prior to a test for CPP expression. We found that CP94253 attenuated the expression of methamphetamine-CPP, but had no effect on acquisition. We harvested the brains 75 min after the test for expression of methamphetamine-CPP in order to examine changes in expression of Fos protein as a marker of transcriptional activity resulting from expression of methamphetamine-CPP. We found that mice expressing methamphetamine-CPP had elevated Fos in the ventral tegmental area (VTA) and basolateral amygdala (BlA) and reduced Fos in the central amygdala (CeA) compared to saline controls. CP94253 given before the expression test, but not acutely in drug-naive mice, enhanced Fos expression in the VTA, nucleus accumbens (NAc) shell and core, and the dorsomedial caudate-putamen, and reversed the methamphetamine-conditioned changes in Fos in the CeA and BlA. Approximately 50-

70% of the Fos in the NAc and VTA was expressed in GABA neurons regardless of group. By contrast, there was no Fos expressed in dopamine neurons in the VTA. The findings suggest that CP94253 attenuates motivational effects of methamphetamine-associated environment and highlight the amygdala, VTA, and NAc as potential regions involved in this effect.

Introduction

Addiction to psychostimulants (i.e., methamphetamine, amphetamine, cocaine) remains a prevalent problem worldwide (NIDA, 2018) and yet there is no effective pharmacological intervention for this disorder. Methamphetamine binds to, and reverses, the dopamine (DA), serotonin (5-HT), epinephrine, and norepinephrine reuptake transporters, causing an increase in synaptic levels of these neurotransmitters (Elliott & Beveridge, 2005; Sulzer et al., 1995; Sager & Torres, 2011; Panenka et al., 2013). While most research on psychostimulants has focused on the role of dopamine in the mesolimbic pathway, serotonin also plays a role in both cocaine and methamphetamine addictive behaviors (for review, see Müller & Homberg, 2015 & Pierce & Kumaresan, 2006), as well as in modulating mesolimbic dopamine neurons (Alex & Pehek, 2007; Van Bockstaele, Cestari, &. Pickel, 1994). One 5-HT receptor subtype found in the ventral tegmental area (VTA) that is known to modulate DA neurons is the 5-HT_{1B} receptor (5-HT_{1B}R; O'Dell & Parson, 2004; Yan et al., 2004). 5-HT_{1B}Rs are widely distributed in the brain (Bruinvels, Palacios, & Hoyer, 1993, Bruinvels et al., 1994; Varnas, Hurd, & Hall, 2005; Clark, McDevitt, & Neumaier, 2006), including in mesolimbic dopamine neurons, which possess both the transcript and protein for 5-HT_{1B}Rs (Bruinvels et al., 1993; Pazos & Palacios, 1985), placing these receptors in the

hallmark addiction-reward pathway. Additionally, single nucleotide polymorphisms (SNPs) in the human 5-HT_{1B}R gene have been shown to be associated with alcohol, cocaine and heroin abuse (Cao, LaRocque, & Li, 2013), aggressive behavior (Hakulinen et al., 2013) attention-deficit/hyperactivity disorder (Smoller et al., 2006) as well as responses to stress and anti-depressants (Mekli et al., 2011; Perroud et al., 2011; Xu et al., 2012).

5-HT_{1B}R agonists facilitate cocaine self-administration in tests occurring during daily access (Parsons et al., 1998); however, following a 21-day period of forced abstinence (post-abstinence), 5-HT_{1B}R agonists decrease cocaine intake (Pentkowski et al., 2009; Pentkowski et al., 2014) and attenuate cocaine-seeking behavior in tests of both cue-induced and cocaine-primed reinstatement occurring after a few weeks of extinction training during which the rats were abstinent (Acosta, Boynton, Kirschner, & Neisewander, 2005; Pentkowski et al., 2009). Furthermore, following a 21-day period of extinction in a cocaine conditioned place preference (CPP) paradigm, CP94253 blocks cocaine-primed reinstatement of extinguished CPP (Der-Ghazarian et al., 2017). These results suggest that pre-abstinence administration of 5-HT_{1B}R agonists facilitates the reinforcing properties of cocaine while post-abstinence 5-HT_{1B}R agonists attenuate the effects (Przegaliński, Gołda, & Filip, 2008).

In contrast to the effects of CP94253 observed with cocaine, we found that this 5-HT_{1B}R agonist attenuates methamphetamine self-administration regardless of whether rats undergo abstinence (Garcia et al., 2017). These findings build on previous research showing similar effects of 5-HT_{1B}R agonists on d-amphetamine self-administration (Fletcher & Korth, 1999; Fletcher et al., 2002; Miszkiel et al., 2012; Miszkiel &

Przegalinski, 2013). Additionally, previous research from our lab and others have shown that CP94253 has no effect on sucrose or food reinforcement (Pentkowski et al., 2009; Przegaliński et al., 2007). Collectively, the findings suggest that 5-HT_{1B}R agonists attenuate intake of amphetamines, without the requirement of abstinence or extinction training.

Cocaine-induced increases in DA mediate the reward learning and habitual behavior that are involved in the development of addiction (Brown et al., 1992; Bunney & Aghajanian 1978; Di Chiara 1998; Grimm et al., 2001; Pettit & Justice 1991; Volkow and Morales 2015). These processes, respectively, involve the mesocorticolimbic DA pathways that originate in the VTA and project to the nucleus accumbens (NAc), prefrontal cortex (PFC), amygdala, and hippocampus (Feltenstein & See, 2008; Pierce & Kumaresan, 2006) and the nigrostriatal DA pathway that originates in the substantia nigra pars compacta and projects to the caudate and putamen (CPu; Wise 2009). 5-HT also plays a complex role in the reinforcing and motivational effects of cocaine and amphetamines (Almalki, Das, Alshehri, Althobaiti, & Sari, 2018; Koe 1976; McFadden, Cordie, Livermont, & Johansen; Woolverton & Johnson 1992; for review, see Cunningham, Bradberry, Chang, & Reith, 1996), which may in part involve modulation of DA mesocorticolimbic and nigrostriatal pathways given that 5-HT neurons originating in the dorsal raphe nucleus project to the substania nigra (SN), CPu, NAc, and VTA (Anden, Dahlstrom, Fuxe, & Larsson, 1965; Fuxe & Ungerstedt, 1968; Hillarp, Fuxe, & Dahlstrom, 1966; Vertes, 1991).

5-HT_{1B}Rs exert an inhibitory effect on neuronal activity via negative coupling with adenylate cyclase, which in turn typically decreases neurotransmitter release (Sari,

2004). The 5-HT_{1B}Rs act either as autoreceptors on 5-HT terminals (Hjorth & Tao, 1991; Sharp, Bramwell, & Grahame-Smith, 1989) or as heteroreceptors on terminals of non-5-HTergic cells (i.e., DA, glutamate, or GABA; Sarhan, Cloez-Tayarani, Massot, Fillion, & Fillion, 1999; Boeijinga & Boddeke, 1996; Chadha, Sur, Atack, & Duty, 2000). Within the VTA, and 5-HT_{1B}Rs act as autoreceptors on 5-HT neuron terminals, as well as heteroreceptors that modulate extracellular GABA and DA in this region (O'Dell & Parson, 2004; Yan et al., 2004). For instance, 5-HT_{1B}R agonist infusion into the VTA or viral overexpression of 5-HT_{1B}Rs inhibits VTA GABA release, and as a result DA neurons in this region are thought to be disinhibited (Nair et al., 2013; O'Dell & Parsons, 2004; Yan et al., 2004). Consistent with this idea, intra-VTA 5-HT_{1B}R agonist infusion increases DA release in the NAc (O'Dell & Parsons, 2004) enhances the development of cocaine sensitization (Przegalinski et al., 2004), and enhances amphetamine-induced hyperlocomotor activity (Papla et al., 2002).

Previous pharmacological and lesion studies have identified several brain regions that play a role in drug-seeking behavior. This brain circuitry involves the basolateral amygdala (BlA; Fuchs et al., 2002, 2005; McLaughlin & See, 2003; Di Ciano & Everitt, 2004) and the central nucleus of the amygdala (CeA; Kruzich & See, 2001; Neisewander et al., 2000), the core of the NAc (NAcC; Di Ciano & Everitt, 2004; Fuchs, Evans, Parker, & See, 2004; Ito, , Robbins, & Everitt, 2004; Kalivas & O'Brien, 2008), the shell of the NAc (Alderson, Parkinson, Robbins, & Everitt, 2001; Bossert, Gray, Lu, & Shaham, 2006; Bossert, Poles, Wihbey, Koya, & Shaham, 2007), dorsolateral CPu (Fuchs, Branham, & See, 2006), the hippocampus (Fuchs et al., 2005), the dorsomedial PFC (McLaughlin and See, 2003; Fuchs et al., 2005), and the VTA (McFarland &

Kalivas, 2001; Neisewander et al., 2000)[for review, see Koob & Volkow, 2010]. Using Fos immunoreactivity as a marker of transcriptional activity, similar regions are involved in the methamphetamine-CPP. Mice expressing methamphetamine-CPP exhibit increases in Fos in the medial PFC (mPFC) and NAcC, but not in the NAc shell (NAcS), dorsomedial CPu (dmCPu), or BlA (Chiang et al., 2009). Similarly, rats exhibiting methamphetamine-CPP show increased Fos in the CPu (Liu et al., 2014). Cocaine CPP experiments have also shown an increase of Fos in the NAcC, prelimbic (PrL), and BlA, but not infralimbic (IL), CeA, CPu in rats expressing cocaine CPP (Miller & Marshall, 2004; Miller & Marshall, 2005a; Miller & Marshall, 2005b).

The brain regions identified using Fos as a marker of brain activity associated with expression of psychostimulant CPP form a neural circuit. For instance, the PrL projects to the BlA (Gabbott, Dickie, Vaid, Headlam, & Bacon, 1997; Gabbot, Warner, Jays, Salway, & Busby, 2005; Vertes 2004) whereas the IL contributes the majority of PFC inputs to the CeA (Hurley, Herbert, Moga, & Saper, 1991; Sesack & Bunney 1989). The PrL and BlA are reciprocally connected and both project to the NAcC (Groenewegen,1988; Groenewegen, Berendse, Wolters, & Lohman, 1990; Maurice, Deniau, Glowinski, & Thierry, 1998). Glutamatergic neurons in the IL projects to the NAcS while those in the PrL project to the NAcC (Phillipson & Griffiths, 1985; Sesack & Grace, 2010; Yager, Garcia, Wunsch, & Ferguson, 2015). Additionally, BlA projects to the NAc (French & Totterdell, 2003; Yu et al., 2017) and receives input from the VTA (Stevenson & Gratton, 2003). Dopaminergic neurons in the VTA innervate the NAcC, NAcS, amygdala, hippocampus, mPFC and ventral pallidum (Carr & Sesack, 1999; Kalivas & Nakamura, 1999; Napier & Maslowski-Cobuzzi, 1994; Stevenson & Gratton,

2003; Sesack, Carr, Omelchenko, & Pinto, 2003; Wise, 2002; Yager et al., 2015). Moreover, the NAcS projects to the VTA (Heimer, Zahm, Churchill, Kalivas, & Wohltmann, 1991). Lastly, the VTA receives projections from the mPFC (Heimer et al., 1997), the dorsal CPu (Watabe-Uchida et al., 2012), and indirectly from the CeA (Geisler, Derst, Veh, & Zahm, 2007; Robbins & Everitt, 2002; for diagram see Fig. 6).

The purpose of this study was to examine the role of 5-HT_{1B}Rs within the circuitry implicated in methamphetamine reward and incentive motivation using the CPP model in mice. First, we examined effects of CP94253 pretreatment on the acquisition methamphetamine-CPP by administering the agonist prior to each methamphetamine amphetamine conditioning session. Second, we examined the effects of CP94253 on the expression of methamphetamine-CPP by administering the agonist prior to the expression test day. Third, to examine brain regions involved in CP94253 effects on incentive motivation for methamphetamine elicited by methamphetamine-paired cues, we examined the effects of CP94253 on Fos protein expression in the VTA, regions of the mPFC, amygdala and NAc, the dmCPu, as well as other interconnected regions. Fourth, we further examined the phenotype of Fos-expressing cells by co-labeling with GAD67 for GABA neurons or TH for DA neurons depending on the brain region. Lastly, as a control procedure we injected a naive group of mice with CP94253 and/or methamphetamine to investigate the effect of the drugs on unconditioned Fos protein expression.

Methods

Animals

Male c57BL/6J mice were obtained from Jackson Laboratories (Sacramento, CA)

and were group housed in a climate-controlled facility with a reversed 10 h light/14 h dark cycle (lights off at 6:00 AM). Mice were handled approximately 1 min/day each of 10 days. Mice in the CPP experiments (n=102; 4 weeks old) were then transferred to single housing so that they would be living alone during CPP procedures, which began the following day. Mice in the control immunohistochemistry experiments (n=30; 6 week old) were group housed and handled daily for at least 10 days prior to harvesting their tissue after acute drug administration as described below. Mice had food and water available *ad libitum* throughout the experiments. All behavioral testing occurred between 8 AM and 4 PM and conditioning procedures took place at the same time of day for a given mouse. Separate groups of mice were used for each specific experiment and were naïve to all experimental manipulations. All husbandry and procedures adhered to the Guide for the Care and Use of Laboratory Animals (2011), and all experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Arizona State University.

Drugs

Methamphetamine hydrochloride (RTI International, Research Triangle Park, NC) and CP94253 (Tocris Bioscience, Minneapolis, MN) were dissolved in saline. All drugs were injected at a volume of 1 ml/100 mg of body weight. We chose doses of methamphetamine that produce CPP in mice according to previous research (Li et al., 2014; Liao et al., 2016; Sun et al., 2016). The dose of CP94253 and the interval between administration for conditioning/testing (30 min) were selected based on previous studies reporting effects on locomotor activity, as well as other behavioral tasks (Bannai et al.,

2007; Der-Ghazarian et al., 2017; Fish et al., 2008; Tatarczynska et al., 2004; Tatarczynska et al., 2005).

Apparatus

CPP experiments were conducted in Plexiglas two-compartment apparatus with each end compartment measuring 35×24×31 cm high and with a removable partition separating them. One compartment had wood chips beneath a wire 1×1 cm grid floor and alternating black and white vertical stripes on the walls. The other compartment had pine bedding beneath a parallel bar floor (5 mm diameter) and alternating black and white horizontal stripes on the walls. In order to prevent the mice from escaping from the chambers while maintaining the ability to record their behavior via an overhanging video camera, a rectangular tower measuring 70×24×74 cm high of clear Plexiglas was used as an extension of the apparatus. The testing room was dimly lit with two overhead lamps, each containing a 25 Watt light bulb. A camera (Panasonic WV-CP284, color CCTV, Suzhou, China) used to record testing sessions was mounted 101 cm above the center of each apparatus. A WinTV 350 personal video recorder (Hauppage, NJ, USA) captured live video encoded into MPEG streams. A modified version of TopScan Software (Clever Sys., Inc. Reston, VA, USA) was used to track body movement. This program uses the orientation of an animal's body parts (e.g. nose, head, center of body, forepaws, base of tail, etc.) to identify the animal's location and specified behaviors.

Experiment 4: Unconditioned effects of acute CP94253 and Methamphetamine on Fos immunohistofluorescence

This experiment investigated effects of acute administration of CP94253 and/or methamphetamine on Fos protein expression, which served as a marker of transcriptional

activity associated with the unconditioned effects of these drugs. These provided a comparison to the changes in Fos expression associated with methamphetamine-CPP in subsequent experiments. Mice (N=14) were first acclimated to handling daily for 10 days. They were then pretreated with either vehicle or CP94253 (10 mg/kg, IP) and 30 min later they were injected with either saline or methamphetamine (3 mg/kg, IP; n/group = 3-5). 90 min after the second injection, mice were perfused while deeply anesthetized with Avertin (300 mg/kg, IP). A 24-gauge needle was inserted into the animal's left ventricle, and the right atrium was clipped with scissors. 15 ml of phosphate buffered saline (PBS) were perfused into the left ventricle, and after exsanguination, 20 ml of 4% paraformaldehyde fixative were administered. After 48 hours in 4% paraformaldehyde, brains were placed into a 15% sucrose solution for a 24-hour period and then transferred to a 30% sucrose solution. After 24 hours, the brains were frozen in OCT compound and stored in the -80°C freezer for future immunohistochemical analysis.

Experiment 5: Effects of CP94253 on the acquisition of methamphetamine-CPP and Fos Immunohistofluorescence

On days 1-3 of this experiment, mice (N=40) were allowed free-access to both sides of the CPP apparatus for 15 min to habituate them to the novel environments. Baseline preference was determined from the average of time spent in each compartment on days 2 and 3. The mice were then assigned to conditioning groups, counterbalanced for baseline preference, that received either vehicle (Veh) or CP94253 (CP; 10 mg/kg, IP) and either saline (Sal) or methamphetamine (METH; 3 mg/kg, IP), resulting in the following 4 groups: Veh+Sal (n=8), CP+Sal (n=12), Veh+METH (n=10), and CP+METH (n=10). Conditioning took place on days 4-7 during a daily, 30-min session.

On days 1 and 4, mice were pretreated with vehicle or CP94253 30 min prior to the start of the conditioning session. Immediately prior to the session, they were given their assigned saline or methamphetamine injection and were placed into their initially non-preferred side for 30 min. On days 2 and 3, mice were injected with vehicle and 30 min later they were injected with saline and placed into their initially preferred side for 30 min. On day 8, mice were given a 15-min preference test for the acquisition of methamphetamine CPP (see Results Fig. 11A for timeline).

Experiment 6: Effects of CP94253 on the expression of methamphetamine-CPP and Fos Immunohistofluorescence

As described above, mice (n=34) were allowed free-access to both sides of the CPP apparatus for 15 min days 1-3 to habituate and to determine baseline preference. On days 4-7, the mice underwent 2 daily, 30-min conditioning sessions separated by a 5-hour period. During the morning session, mice were injected with saline and placed into their initially preferred side, and during the afternoon session, mice were injected with methamphetamine (1 mg/kg, IP) and placed into their initially non-preferred side. On days 8 and 11, mice were tested for the expression of methamphetamine CPP for 15 min (see Results Fig. 11B for timeline). Mice that failed to express methamphetamine-CPP on day 8 were eliminated from the study. On day 11, mice were pretreated with either saline (METH+Sal Group) or CP94253 (METH+CP Group; 10 mg/kg, IP) 30 min prior to the 15-min test. Additionally, a control group of mice (n=10) that had received saline prior to each conditioning session were given a vehicle pretreatment, and 30 min later received saline immediately prior to testing (Veh+Sal Group). 75-min after the conclusion of the 15 min expression test, mice underwent perfusion and their brains were harvested and

stored as described in Experiment 4 (note that the total amount of time that elapsed from the CP94253/vehicle injection until perfusion was 120 min).

Experiment 7: Effects of acute CP94253 on Fos Immunohistofluorescence

We further verified unconditioned effects of acute CP94253 in this experiment. A separate cohort of mice that had been acclimated to handling were injected with either saline (n=8) or CP94253 (10 mg/kg, IP; n=8). 120 min later, their brains were harvested after perfusion and were stored at -80ºC as described for Experiment 4.

Fos Immunohistofluorescence

The brains were sectioned at 40 μm in the coronal plane using a cryostat (Leica CM1860) maintained at -18ºC. Sections were collected at anatomical locations corresponding to levels +1.41 mm, +1.09 mm, -1.23 mm, and -3.07 mm from bregma as shown in Fig. 7 (Paxinos & Watson, 2013). Later, sections were rinsed with phosphate buffered saline (PBS) three times (15 min each) and then were incubated with 5% normal donkey serum (NDS) in 0.2% Triton X-100 in PBS (NDS/PBST) for 30 min at room temperature. Primary antibodies were diluted to the appropriate concentration in 5% NDS/PBST solution prior to applying them to the sections, which were then incubated for 2 days at 4°C with gentle agitation. After rinsing in PBST three times (15 min each), the secondary antibody diluted in 5% NDS/PBST was added and sections were incubated for 2 days at 4°C. After 48 hours, sections were rinsed 3 times with 0.2% PBST and mounted onto Fisher Selectfrost slides and a cover slip was applied using Vectashield HardSet antifade mounting medium with DAPI (H-1500, Vector Laboratories, Burlingame, CA). The antibodies utilized were chicken anti-tyrosine hydroxylase (1:1000 dilution; ab76442, Abcam, Cambridge, MA) with secondary antibody Alexa Fluor 647, goat anti-

c-fos (1:1000 dilution; sc-52G, Santa Cruz Biotechnology, Santa Cruz, CA) with secondary antibody Alexa Fluora 488, and rabbit anti-GAD1/GAD67 (1:1000 dilution; 198 013, Synaptic Systems, Germany) with secondary antibody Alexa Fluora 555. All secondary antibodies were conjugated donkey at 1:1000 dilution (ThermoFisher Scientific; Waltham, MA).

Immunoreactivity Analysis

Images were taken at 20x magnification with a Zeiss LSM800 laser scanning confocal microscope (Experiment 4 & Experiment 6) or an Olympus BX53 epifluorescence microscope (Experiment 7). The regions imaged were determined using a mouse brain atlas (Paxinos & Franklin, 2013) as illustrated in Fig. 7 and 9. Sections taken at +1.41 mm from bregma contained the PrL and IL regions of the medial prefrontal cortex (mPFC), the nucleus accumbens shell (NAcS), and nucleus accumbens core (NAcC). Sections taken at +1.09 mm from bregma included the dorsal medial caudate-putamen (dmCPu). Sections taken at -1.23 mm from bregma contained the basolateral amygdala (BlA) and central nucleus of the amygdala (CeA). Sections taken at −3.07 mm from bregma included the ventral tegmental area (VTA). In experiment 4, 1-2 tissue sections from one hemisphere from were imaged. For experiment 6, each region was analyzed using two tissue sections from one hemisphere of each animal. In experiment 7, three tissue sections including both hemispheres were imaged for each region. Occasionally, a sample was omitted due to artifacts and such decisions were made blind to the experimental group assignment. For all of the experiments, sample values from replicate sections of a region were averaged and the average value was used in the analyses. Images were optimized for brightness and contrast in photoshop by an

experimenter blind to the condition. The region of interest was then outlined manually in Photoshop and Fos immunoreactive cells were counted blind to treatment conditions. We determined the density of Fos and GAD67+Fos per mm^2 in each analyzed region. To calculate the percent of Fos cells co-labeled with GAD67, we divided number of Fos cells co-labelled with GAD67 by the total number Fos labelled cells.

Statistical Analysis

Fos-positive nuclei, time spent in the initially non-preferred side of the CPP apparatus, and rate of activity in each side of the apparatus were analyzed using ANOVAs with Pretreatment (Vehicle or CP94253) and Conditioning Treatment (Saline or METH) or Group as between group factors and Day (average baseline, CPP test, expression test) as a repeated measure where appropriate. Interactions were analyzed by smaller ANOVAs and t-tests with Bonferroni corrections where appropriate.

Results

Experiment 4: Unconditioned effects of acute CP94253 and Methamphetamine on Fos immunohistofluorescence

The unconditioned effects of acute CP94253 (10 mg/kg) and/or methamphetamine (3 mg/kg) administration on Fos protein expression were examined in the cingulate cortex, NacC, and NAcS, BlA, CEA, hippocampus CA1 and CA3 regions, and VTA. An ANOVA with pretreatment (vehicle or CP94253) and treatment (saline or METH) as between subjects factors revealed a treatment main effect (see Fig. 8) in the VTA [$F(1, 11) = 5.34$, $p<0.05$] and dmCPu [$F(1, 11) = 35.58$, $p<0.001$]. In both of these regions, mice that received 3 mg/kg methamphetamine, regardless of pretreatment injection, had greater Fos expression than mice receiving saline. In the CeA, there was a

main effect of pretreatment [F(1, 10) = 89.43, p<0.001], a main effect of treatment [F(1, 10) = 97.53, p<0.001] and an interaction between the two variables [see Fig. 8 and 10; F(1, 10) = 56.40, p<0.001]. Mice that received both CP+METH showed increased Fos expression when compared to all other groups. There were no effects of the drugs on Fos expression in the hippocampus CA1 and CA3 regions, the cingulate cortex, the BlA, or NAcC, or NAcS. Thus, it appears that CP94253 enhances unconditioned Fos expression in the CeA and that this effect is potentiated by methamphetamine.

Verification of unbiased apparatus

Across experiments in this study, approximately ~40% of the mice preferred the side of the apparatus with horizontal stripes and ~60% preferred the side with vertical stripes, confirming the use of an unbiased apparatus. To the extent possible, mice were distributed among treatment groups counterbalanced for initial side preference and magnitude of initial side preference.

Experiment 5: CP94253 has no effect on acquisition of methamphetamine-CPP

The timeline for Experiment 2 is shown in Fig. 11A. The overall ANOVA of time spent in the initially nonpreferred side including Pretreatment, Conditioning Treatment, and Day revealed strong main effects of Day [F(1,36)=70.0, p<0.0001] and Conditioning Treatment [F(1,36)=7.72, p<0.01] that likely obscured detecting a 3-way interaction. To further examine potential group differences, a simpler ANOVA with Day and Group (all four groups) as factors was conducted. This analysis revealed a significant Day main effect [F(1,36) = 70.0, p<0.001] and a Day X Group interaction [F(3,36) = 3.61, p<0.05; Fig. 12A]. Subsequent paired t-tests with Bonferroni correction showed a significant increase in time spent in the initially nonpreferred (i.e., drug-paired) side of the apparatus

on the test day compared to baseline for the CP+Sal [t(11)=4.93, p<0.001], the Veh+METH [t(9)=5.06, p=0.001], and the CP+METH [t(9)=8.61, p<0.001] groups, whereas there was no change across days in the Veh+Sal group. Furthermore, one-way ANOVAs of time spent in the initially nonpreferred side on the baseline test day showed no group differences [F(3,36)=1.19], whereas on the test day there was an effect of group [F(3,36)=3.69, p<0.05]. Comparisons to the Veh+Sal control group showed a difference only from the CP+METH group [t(16)=3.71, p<0.01], although there was a trend toward a difference from the Veh+METH group as well [t(16)=2.44, p=0.027, not significant with Bonferroni correction]. Collectively, these findings suggest that all drug-treated groups showed a shift in preference, however, the magnitude of preference shift was slightly higher in the methamphetamine-conditioned groups. CP94253 did not alter the acquisition of methamphetamine-CPP.

We also examined locomotor activity on the CPP test day (see Fig. 12B). Because mice spent varying amounts of time on each side, we calculated a locomotor activity rate (mm traveled/sec + SEM) by dividing distance travelled in the drug-paired or saline-paired side of the apparatus by the total time spent in that respective side during the test. The overall ANOVA of activity rate revealed an effect of Conditioning Treatment [F(1,36)=5.09, p<0.05], indicating that methamphetamine-conditioned groups exhibited lower activity rates.

Experiment 6a: CP94253 blocks the expression of methamphetamine-CPP

The timeline for Experiment 3 is shown in Fig. 11B. We used a methamphetamine dose (1 mg/kg, IP) that would produce a relatively weak methamphetamine-CPP so that we would have the sensitivity to attenuate or enhance

CPP expression by pretreatment with CP94253. Methamphetamine-conditioned mice that failed to meet the acquisition criterion of ≥450 s spent in the drug-paired side on the test day were eliminated such that only mice exhibiting CPP were tested for CP94253 effects on CPP and Fos expression. Mice meeting the criterion were further divided into groups that received either vehicle or CP94253 prior to the second test, counterbalanced for the magnitude of their initial CPP expression. The final n/group ranged from 10-12. A repeated measures analysis of time spent in the initially nonpreferred side across the tests showed a significant Day main effect [$F(2, 62)=44.31$, $p<0.001$], a Group main effect [$F(2, 31)=13.93$, $p<0.001$], and a Day x Group interaction [$F(4, 62)=5.96$, $p<0.001$; Fig. 13A]. To further analyze the interaction, we performed subsequent one-way ANOVAs across Day for each group. As expected, there were no differences across tests in the Sal+Veh group. There was an effect of Day in the METH+Veh group [$F(2, 22)=49.41$, $p<0.001$], and subsequent paired-samples t-tests with Bonferroni correction showed an increase in time spent on the initially nonpreferred side on both the CPP test and the expression test compared to the baseline test [$t(11)=11.24$, $p<0.001$; $t(11)=8.78$, $p<0.001$, respectively]. Lastly, there was also an effect of Day in the METH+CP Group [$F(2, 22)=22.52$, $p<0.001$]. Subsequent paired-samples t-tests showed an increase in time spent on the initially nonpreferred side on the CPP test compared to the baseline test [$t(11)=9.11$, $p<0.001$], but no significant increase on the expression test. These findings are consistent with our hypothesis that stimulation of 5-HT_{1B}Rs with the agonist CP94253 attenuates the expression of methamphetamine-CPP.

The locomotor activity rate (mm traveled/sec + SEM) was calculated as previously stated. Analysis of locomotion on the initial CPP test day indicated that mice

conditioned with 1 mg/kg methamphetamine travelled less distance than saline control mice [Conditioning main effect $F(1,32)=4.57$, $p<0.05$; see Fig. 13B]. On the expression test day, there were also significant differences in distance travelled across groups [$F(2, 31)=5.67$, $p<0.01$; see Fig. 13C]. Post hoc t-tests with Bonferroni correction showed that methamphetamine-conditioned mice receiving CP on the expression test day exhibited higher distance travelled than the control Sal+Veh group ($p<0.01$). Within the methamphetamine-conditioned groups there was no significant difference in distance travelled during the expression test.

Experiment 6b: Methamphetamine conditioning and CP94253 effects on Fos Immunohistofluorescence

The results of Fos immunohistochemistry in mice tested for expression of methamphetamine-CPP with and without CP94253 pretreatment is shown in Fig. 14. One-way ANOVAs for each region analyzed revealed differences across groups in the VTA [($F(2, 23) = 5.68$, $p=0.01$]; dmCPu [($F(2, 23) = 73.95$, $p<0.001$]; NAcS [($F(2, 19) = 34.7$, $p<0.001$]; NAcC [($F(2, 19) = 15.96$, $p<0.001$]; CeA [($F(2, 25) = 3.69$, $p<0.05$]; and BlA [($F(2, 24) = 4.29$, $p<0.05$]. There were no changes in Fos expression in the cortical regions examined, although there was a trend toward differences across groups in the PrL ($p=.083$; see Fig. 14G). Methamphetamine conditioning effects on Fos expression were observed as an increase in the METH+Veh compared to the Sal+Veh controls in the VTA [$t(16)=2.80$, $p=0.014$] and BlA [$t(17)=2.71$, $p=0.015$], but a reduction in the CeA [$t(17)=2.73$, $p=0.014$]. CP94253 did not alter methamphetamine-conditioned Fos expression in the VTA as the METH+CP did not differ from the METH+Veh group but was different from the control group [$t(14)=3.99$, $p=0.001$];

however, the methamphetamine-conditioned increase in Fos in the BlA and decrease in Fos in the CeA appeared to be attenuated by CP94253 pretreatment as that METH+CP group did not differ from either the Sal+Veh or the METH+Veh groups. CP94253 alone increased Fos expression compared to both the Sal+Veh and METH+Veh groups in striatal subregions, including the dmCPu [t(14)=9.10, $p<0.001$; t(16)=9.95, $p<0.001$], NAcC [t(12)=4.62, $p=0.001$; t(13)=4.52, $p=0.001$], and NAcS [t(12)=3.46, $p=0.01$; t(13)=3.38, $p<0.01$]. Representative fluorescent images of Fos for the CeA, NAcS, and VTA are in Fig. 15.

Additionally, we examined co-labelling of Fos with GAD67 or TH depending on the brain region. We observed no co-localization of Fos with TH in the VTA. Approximately 70% of the Fos-labelled cells in the VTA co-labeled with GAD67, although there were no group differences in the number of co-labeled cells across groups (see Table 1 and Fig. 16). We further examined GAD67 and Fos co-labelled cells in the other brain regions and observed a range of co-labelling across these regions (see Table 1), however, again there were no group differences in the percentage of co-labeled cells (data not shown).

Experiment 7: Verification of unconditioned effects of CP94253 on Fos protein expression

The results of our final experiment confirmed that acute exposure to CP94253 produced an unconditioned increase in Fos expression in the CeA [$t(14)=2.38$, $p<0.05$; see Fig. 17E]. We found no significant differences in any of the other brain regions analyzed.

Discussion

This study found that the 5-HT_{1B}R agonist CP94253 attenuated the expression of methamphetamine-CPP in mice (Fig. 12). Specifically, the expression of methamphetamine-CPP that was established with four pairings of 1 mg/kg methamphetamine with the initially nonpreferred environment was blocked in mice pretreated with CP94253 (10 mg/kg, IP) prior to the preference test. The learned association between cues and the rewarding effects of psychostimulant drugs of abuse is an important component of human drug relapse and craving. Drug seeking in addicts often depends on the association formed between drug-paired cues and the rewarding effects of the drug. Methamphetamine-conditioned mice spent more time in the drug-paired environment (i.e., cues), indicating that the mice were motivated to seek the environment which previously predicted drug-reward because the cues in that environment had acquired incentive motivational value. It is likely that CP94253 attenuated the incentive motivational effect of the environmental cues, thereby blocking the expression of CPP. Consistent with this interpretation, Garcia and colleagues found that CP94253 attenuates methamphetamine intake on a progressive ratio schedule when administered both during maintenance of self-administration and after abstinence (Garcia et al., 2017). Progressive ratio is a high effort schedule that measures reinforcement, but is also sensitive to motivation to seek the reinforcer. Previous studies also report that 5-HT_{1B}R agonists attenuate d-amphetamine intake on a progressive ratio schedule, suggesting CP94253 reduced incentive motivation to seek the drug (Fletcher & Korth, 1999; Fletcher et al., 2002; Miszkiel et al., 2012; Miszkiel & Przegalinski, 2013). Therefore, it is not surprisingly that CP94253 blocked the expression of methamphetamine-induced CPP, likely by reducing the incentive motivational effect of

the conditioned environmental cue to elicit drug seeking.

By contrast, CP94253 did not block the acquisition of methamphetamine-CPP, as CP94253 given prior to conditioning sessions had no effect on CPP established with 2 pairings of 3 mg/kg methamphetamine with the initially nonpreferred compartment (Fig. 11). The lack of effect of CP94253 on acquisition suggests that the rewarding effects of methamphetamine were unaltered. Furthermore, this finding suggests that CP94253 did not affect learning and memory as evidenced by the formation of a drug-compartment association. The lack of CP94253 effect on acquisition is surprising, given that Garcia and colleagues (2017) found that CP94253 reduced the reinforcing effects of methamphetamine using a VR5 schedule, and drug reward is a key component of reinforcement. It is possible that acquisition of methamphetamine-CPP may be attenuated when using other parameters (i.e., different methamphetamine doses and/or conditioning schedules) or that 5-HT_{1B}R stimulation becomes critical for reinforcement in animals with an extensive history of drug exposure as occurred in the Garcia et al. study.

Other possible reasons for the 5-HT_{1B}R agonist attenuation of methamphetamine-induced CPP expression include an effect on motor capability or anxiety. Impairment in motor capability seems unlikely because mice receiving CP94253 exhibited increased locomotor activity compared to the saline control group and did not differ from the methamphetamine-conditioned group that displayed CPP. Furthermore, previous research has shown that CP94253 has no effect on sucrose or food reinforcement, which rely on performing an operant response (Pentkowski et al., 2009; Przegaliński et al., 2007). It is more difficult to ascertain whether anxiety contributed to the CP94253 blockade of methamphetamine-CPP expression because the role of 5-HT_{1B}Rs in anxiety is complex.

5-HT_{1B}R KO mice have a phenotype that demonstrates reduced anxiety and increased aggression (Gingrich & Hen 2001, Groenink, van Bogaert, van der Gugten, Oosting, & Olivier, 2003, Guilloux et al 2011; Zhuang et al., 1999). However, experiments with 5-HT_{1B}R KO mice produce conflicting results on anxiety levels, with some studies reporting no change (Brunner, Buhot, Hen, & Hofer, 1999; Malleret, Hen, R., Guillou, Segu, & Buhot, 1999; Sibille et al., 2007) and others reporting reduced anxiety (Zhuang et al., 1999; Bouwknecht et al., 2001a). In wild type mice, CP94253 can have anxiolytic and antidepressant-like effects (Tatarczyńska et al., 2004; Tatarczyńska et al., 2005). In rats, 5-HT_{1B}R agonists or antagonists can increase baseline anxiety levels, as well as cocaine-induced anxiety-like behaviors (Lin & Parsons, 2002; Hoplight et al., 2005; Pentkowski et al., 2009). Over-expressing 5-HT_{1B}Rs in dorsal raphe nucleus (DRN) projection neurons produces anxiety-like behavior in the plus maze and open field tests, but only after a stress-inducing procedure (Clark et al., 2002). Along this line of reasoning, stress often motivates drug-seeking and increases drug intake (Goeders & Guerin, 1994; Ahmed & Koob, 1997; Piazza & Le Moal, 1998; Logrip, Zorrilla, & Koob, 2012). Thus, the stress associated with a CP94253-induced increase in anxiety would be expected to enhance expression of CPP rather than attenuate expression as observed in this study.

The effects of CP94253 on psychostimulant behaviors vary across cocaine versus amphetamines. Although post-abstinence this agonist attenuates both methamphetamine and cocaine self-administration reinforcement (Garcia et al., 2017; Pentkowski et al., 2012; Pentkowski et al., 2014), when given prior to abstinence CP94253 enhances cocaine reinforcement but attenuates methamphetamine reinforcement (Pentkowski et al.,

2012; Pentkowski et al., 2014). Both methamphetamine and cocaine inhibit 5-HT, dopamine, and norepinephrine transporters and cause a down-regulation of these transporters with repeated use (Azzaro & Rutledge, 1973; Ritz, Cone, & Kuhar, 1990). However, they interact differently with the transporters. Cocaine inhibits monoamine transport back into the cell. In addition to this action, methamphetamine also redistributes intracellular monoamines by acting at the vesicular monoamine transporter (VMAT) which not only causes the release of monoamines into the cytosol but also reverses monoamine transport across the plasma membrane resulting in more monoamine release in the cytosol (Sulzer et al., 1995; Sager & Torres, 2011; Panenka et al., 2013). Additionally, cocaine and amphetamines produce differential effects on the releasable vesicular pool and on regulation of VMAT-2 (Brown, Hanson, & Fleckenstein, 2001). Specifically, cocaine increases VMAT-2 activity while in contrast, methamphetamine reduces VMAT-2 function (Brown et al., 2001). Lastly, the effects of cocaine are more dependent on neurotransmitter tone in the synapse than amphetamines. This may contribute to the paradoxical effects seen with CP94253 during pre-abstinence vs. post-abstinence.

Multiple manipulations in the mesolimbic system have shown that 5-HT_{1B}Rs may play a modulatory role in psychostimulant addiction (Neumaier et al., 2002; Filip, Papla, Nowak, Jungersmith, & Przegaliński, 2002; Pentkowski et al., 2012; Papla et al., 2002; Przegaliński et al., 2002; Przegaliński et al., 2004). The lead hypothesis for 5-HT_{1B}R modulation of psychostimulant effects is that 5-HT_{1B}Rs on GABA interneurons or GABA terminals in the VTA of medium spiny neurons from the NAc modulate DA neuron activity (O'Dell & Parsons 2004; Yan et al., 2004). We explored the circuitry

further in this study using Fos as a marker of transcription regulation associated with neural processing in response to acute stimulus conditions. Specifically, we investigated both the unconditioned and conditioned effects of CP94253 and methamphetamine on Fos protein expression.

In assessing the unconditioned effects of acute CP94253 and/or methamphetamine (Experiments 4 and 7), we found that CP94253 alone increased Fos in the CeA only (see Fig. 8 and 16) and that acute methamphetamine increased Fos in the dmCPu, VTA, and CeA (see Fig. 8). Interestingly in the CeA, CP94253 interacted with methamphetamine, resulting in a robust increase in Fos expression that was elevated compared to all other groups (see Fig. 8). This is consistent with a previous study showing increased Fos in the CeA but not the BlA after an acute injection of 20 mg/kg CP94253 in mice (Lee, Somerville, Kennett, Dourish, & Clifton, 2004).

In assessing the conditioned effects of methamphetamine and their modulation by CP94253, we found that mice expressing methamphetamine CPP showed increased Fos protein expression in the VTA and BlA, and a reduction in the CeA in contrast to the unconditioned increase in this region (Fig. 13). In mice showing CP94253-attenuated expression of methamphetamine-CPP, Fos protein expression was elevated in the dmCPu, NAcS, and NAcC compared to both saline controls and the methamphetamine-conditioned group (Fig. 13). CP94253 did not affect the methamphetamine-conditioned increase in Fos in the VTA, although there was a trend toward enhancement of this effect (Fig. 13). Additionally, CP94253 reversed the methamphetamine-conditioned decrease in Fos in the CeA and the methamphetamine-conditioned increase in the BlA. Given that acute CP94253 treatment in unconditioned controls from experiments 4 and 7 only

showed increased Fos in the CeA, the CP94253-induced increases in Fos observed in the dmCPu, NAcS, and NAcC of methamphetamine-conditioned mice may be related to processes involved in CP94253-induced attenuation of methamphetamine-CPP.

The increased Fos observed in the VTA in mice expressing methamphetamine-CPP was anticipated given that the VTA is a region important for reward-seeking behaviors, especially when these behaviors are triggered by Pavlovian cues (Roitman, Stuber, Phillips, Wightman, & Carelli, 2004; Yun, Wakabayashi, Fields, & Nicola, 2004; Corbit, Janak, & Balleine, 2007; Kufahl et al., 2009; Zijlstra, Veltman, Booij, van den Brink, & Franken, 2009). The VTA contains dopamine (DA) and non-DA neurons, and both are involved in the reinforcing and motivational effects of other natural rewards as well as drugs (Wise & Bozarth, 1981; Ikemoto & Panksepp, 1999; Salamone, Correa, Farrar, & Mingote, 2007; Schultz, 2010). Given the hypothesized role of the VTA GABA neurons in the modulatory effects of 5-HT_{1B}R agonists on cocaine behavioral effects, we hypothesized that methamphetamine CPP expression involved increased signaling in DA neurons that would be evident as an increase in Fos. Surprisingly, we found that none of the Fos co-localized with TH in dopamine neurons, but approximately 70% of the Fos co-localized with GAD67. Furthermore, methamphetamine-conditioned Fos expression was further enhanced in the VTA by CP94253, contrary to our expectation that CP94253 stimulation of 5-HT_{1B}R heteroreceptors on GABA neurons would inhibit these cells in the VTA, resulting in reduced Fos expression. It is possible that the increased Fos expressed in the CP94253-treated, methamphetamine-conditioned mice occurs via disinhibition of GABAergic interneurons by CP94253-inhibited GABA afferent neurons to the interneurons. VTA GABA neurons are responsive to reward-predictive cues

(Brown et al., 2012; Cohen, Haesler, Vong, Lowell, & Uchida, 2012) and their activation is sufficient to disrupt reward consumption or induce avoidance behavior (Tan et al., 2012; van Zessen, Phillips, Budygin, & Stuber, 2012). Activation of these GABAergic neurons may explain the CP94253-induced attenuation of methamphetamine-CPP and increase in Fos. It is also possible that CP94253 inhibited GABA neurons that released efferent glutamate neurons from inhibition given that 30% of Fos positive neurons in the VTA were not co-labeled with either TH or GAD67 and research has shown that the VTA also contains glutamatergic neurons (Dobi, Margolis, Wang, Harvey, & Morales 2010; Hnasko, Hjelmstad, Fields, & Edwards 2012; Yoo et al., 2006). Indeed, vesicular glutamate transporter 2 (VGLUT2)-labeled glutamate neurons comprise approximately up to 35% of NAc-projecting neurons in VTA (Nair-Roberts et al., 2008; Yamaguchi, Wang, Li, Ng, & Morales, 2011). Research on the glutamatergic neuronal population in the VTA and its contribution to reward and motivation to seek psychostimulants are scarce. Further investigation of the sub-populations of VTA neurons expressing Fos in response to CP94253 is needed.

Surprisingly, we did not observe Fos changes in the NAc in methamphetamine-conditioned mice whilst previous research has found cocaine- or methamphetamine-CPP expression is associated with increased Fos in the NAcC or NAcS in mice and rats (Chiang et al., 2009; Miller & Marshall 2004; Miller & Marshal, 2005a; Miller & Marshall, 2005b). It is important to note that our expression paradigm differed from previous research as we had two CPP preference tests (i.e., CPP test and Expression test) separated by 72 hours, not a single preference test. Given the repeated exposure to the same stimulus (i.e., test chamber), Fos expression in methamphetamine-conditioned mice

likely showed tolerance and it is possible that CP94253 activated pathways involved in inhibiting CPP rather than pathways involved in the expression of CPP. Psychostimulants are directly self-administered into the NAcS and produce a CPP when injected into this region (McBride, Murphy, Ikemoto, 1999). The NAcS appears to be involved in the rewarding effects of drugs of abuse (Ito et al., 2004; Sellings & Clarke, 2003) and in suppressing cocaine seeking after extinction (Peters, LaLumiere, & Kalivas, 2008). The NAcC is critical for initiating cocaine seeking (Kalivas & O'Brien, 2008), is required for maintaining cue-elicited drug-seeking behavior (Fuchs et al., 2004; Ito et al., 2004; Di Ciano & Everitt, 2004), and lies at the interface of motivation and movement (Mogenson, Jones, & Yim, 1980).

Although no conditioned Fos expression was observed in the NAcC and NAcS, acute administration of CP94253 prior to the test for expression of CPP increased Fos expression in these regions. Thus, mice that expressed CPP on the test day showed no increase in Fos in the NAc, whereas mice that did not express CPP due to CP94253 pretreatment showed an increase in Fos. The CP94253-induced Fos in these regions is not an unconditioned effect of CP94253 as shown in Experiments 1 and 4, but rather is an effect that only occurs in previously conditioned mice. Previous research has shown that activation of 5-HT_{1B}Rs in the NAc decreases the rewarding and reinforcing effects of amphetamine (Fletcher & Korth, 1999; Fletcher, 2002). Furthermore, local activation of 5-HT_{1B}Rs in the ventral tegmental area potentiates cocaine-induced increases in dopamine levels in the NAc and cocaine-induced decreases in GABA (Parsons et al., 1999; O'Dell & Parsons, 2004). Therefore, it is possible that the attenuation of methamphetamine-CPP may have been achieved by 5-HT_{1B}R agonism with CP94253 in

the NAcC, NAcS, and VTA, by blocking incentive motivation to seek the drug. In both the NAcC and NAcS approximately 50% of the expressed Fos co-localized with GAD67 indicating that a large population of inhibitory neurons were activated by CP94253. Perhaps a functional consequence of the enhanced intracellular signaling in these GABA neurons that caused Fos expression is the attenuation of methamphetamine-CPP.

We observed no changes in Fos expression in either the PrL or IL in methamphetamine-conditioned mice regardless of CP94253 pretreatment. It is surprising that methamphetamine-CPP did not affect Fos expression in the PrL because previous studies have shown that mice and rats expressing cocaine or methamphetamine-CPP exhibit increased Fos in the PrL or mPFC (Chiang et al., 2009; Miller & Marshall 2004; Miller & Marshal, 2005a; Miller & Marshall, 2005b). This region is critical for initiating cocaine seeking along with the NAcC (Kalivas & O'Brien, 2008) and contributes to executive decision-making processes of response initiation and inhibition (Bechara, Tranel, & Damasio, 2000; Iversen & Mishkin, 1970; Weissenborn, Robbins, & Everitt, 1997). On the other hand, the IL suppress cocaine seeking after extinction along with the NAcS (Peters et al., 2008) and has been shown to be critically important in extinction of Pavlovian fear conditioning, controlling addiction-seeking behavior, and habit formation (Barker, Taylor, & Chandler, 2014; Gutman et al., 2017; Killcross & Coutureau, 2003; Laurent & Westbrook, 2009; Peters, Kalivas, & Quirk, 2009; Sangha, Robinson, Greba, Davies, & Howland, 2014; Smith, Virkud, Aeisseroth, & Graybiel, 2012). We had expected to observe a conditioned increase in Fos expression in the PrL, but not IL. The lack of effect in methamphetamine-conditioned mice may have been due to the repeated testing procedure as discussed above. The lack of a CP94253-induced Fos

expression in methamphetamine-conditioned mice, in contrast to the increases observed in striatal regions, suggests that 5-HT_{1B}R modulation of CPP expression may occur in striatal regions rather than in the cortex.

Lastly, we observed an increase of Fos in the BlA in methamphetamine-conditioned mice which is not surprising given the BlA is the main input region of stimuli and is responsible for the modulation and processing of emotional memories (Cahill & McGaugh, 1998; Koob, 2008), memory consolidation (Pare, 2003), associative learning (Everitt et al., 1999; LeDoux, 2000), and has been implicated in processing and modifying the incentive motivational value of drug-associated contextual and discrete cues (Everitt et al., 1999; Fuchs & See 2002; Fuchs, Weber, Rice, & Neisewander., 2002; Grimm & See 2000; McLaughlin & See, 2003). Methamphetamine-conditioned mice showing enhanced Fos in the BlA on expression test day may reflective of the incentive motivational value of the drug-paired chamber. After evaluating the emotional valence of stimuli, the BlA sends projections to the CeA that is thought to organize the behavioral response and reinforce behavior (Fuchs & See 2002; Grimm & See 2000; LeDoux, 2000). We found decreased Fos in the CeA of methamphetamine-conditioned mice. Given the role the CeA plays in novelty-seeking and exploratory behavior and that methamphetamine-conditioned mice were not exploring both chambers, there perhaps was reduced activation of the CeA due to the reduced need to organize behavioral output and engage in exploratory behavior.

Specifically, the amygdala is thought to underlie Pavlovian learning in which outcomes are predicted by sensory cues (Baxter & Murray, 2002; Hampton, Adolphs, Tyszka, & O'Doherty, 2000; LeDoux 2000; Seymour & Dolan, 2008; Wassum &

Izquierdo, 2015). Additionally, the BlA receives major serotonergic projections from the DRN (Davis 1992; Herry et al., 2010; Johansen et al., 2010; Lowry et al., 2005); densest to the BlA, weaker in the CeA (Vertes 1991). This is indicative of serotonin in the amygdala and presumably its involvement in amygdala-mediated behaviors. Previous research investigating Fos in the BlA in mice expressing methamphetamine-CPP did not see an increase (Chiang et al., 2009). However, in rats expressing cocaine CPP, there was increased Fos expression in the BlA and no change in Fos in the CeA (Miller & Marshall 2004; Miller & Marshall 2005a), even though we found a reduction of Fos in the CeA in methamphetamine-conditioned mice. The discrepancies in the data can again be a result of different CPP testing procedures.

CP94253 reduced the increase in Fos observed in the BlA of methamphetamine-conditioned mice which reflects a decrease in the motivational value of the meth-paired context which likely dampened the motivation to seek the conditioned reward (methamphetamine). CP94253 reversed the reduction of Fos observed in the methamphetamine-conditioned mice. It is possible the modulation of neuronal activity in the CeA by CP94253 disrupted the motivational value of the conditioned environment resulting in more exploratory behavior. In our study less than 20% of the Fos in the BlA co-localized with GAD67 indicating a role for glutamatergic neuronal population which is not surprising given that the principle output neurons of the BlA are glutamatergic (80%–90%). The methamphetamine-conditioned increase of Fos observed in the BlA, presumably in glutamatergic neurons, likely manifested by the emotional salience of methamphetamine and the drug-paired chamber. Although the majority (95%) of CeA neurons are GABAergic, the CeA receives glutamatergic input from the BlA (Krettek &

Price, 1978; Pitkanen et al., 1995; Savander, Go, LeDoux, & Pitkanen, 1995) and projects to the bed nucleus of the stria terminalis (BNST) and VTA. In our study, 64% of the Fos co-localized with GABAergic neurons in the CeA indicating a potential role for other neuronal subtypes.

The present findings suggest an inhibitory role of 5-HT_{1B}Rs in the motivational effects of methamphetamine-paired cues given CP94253 attenuated the expression of methamphetamine-CPP in mice. These findings build upon previous research using rats demonstrating 5-HT_{1B}R agonists reduce incentive motivation for d-amphetamine in a self-administration model (Fletcher & Korth, 1999; Fletcher et al., 2002; Miszkiel et al., 2012; Miszkiel & Przegalinski, 2013). CP94253 did not block the rewarding effects of methamphetamine nor the learned association between environmental cues and methamphetamine as CP94253 did not block the acquisition of methamphetamine-CPP. In methamphetamine-conditioned mice, CP94253 reversed the increased Fos observed in the BlA and the decreased Fos observed in the CeA. CP94253 also increased Fos in the VTA, NAcS, and NAcC of methamphetamine-conditioned mice. Acute CP94253 only increased Fos in the CeA indicating that the pattern of Fos observed in methamphetamine-conditioned mice may be related to processes involved in CP94253-induced attenuation of methamphetamine-CPP. Although the specific mechanisms responsible for the attenuating effect of 5-HT_{1B}R agonists on methamphetamine-induced CPP are unclear, we hypothesize that such mechanisms may involve the 5-HT_{1B}Rs in the NAc, VTA, and amygdala circuitries as evidenced by our Fos data. Collectively, we postulate that the pattern of Fos activation in these regions reduces the incentive motivation to seek the methamphetamine-paired environment. Important future directions

include deciphering the sub-neural circuitry involved in the agonist effects. Lastly, Garcia et al., 2017 showed that zolmitriptan, FDA-approved 5-$HT_{1D/1B}$R agonist, decreased methamphetamine intake when given acutely during maintenance, as well as given intermittently following abstinence. Given that the anti-migraine medication zolmitriptan is clinically available, 5-HT_{1B}R agonists warrant further investigation as possible treatments for psychostimulant addiction.

CHAPTER 4

CONCLUDING REMARKS

This dissertation aimed to test the hypothesis that 5-HT_{1B}Rs modulate cocaine and methamphetamine abuse-related behaviors in mice. This hypothesis was examined using the 5-HT_{1B}R agonist CP94253 to stimulate 5- HT_{1B}Rs. The main findings that support the hypothesis demonstrate that CP94253: 1) attenuated the expression of cocaine-sensitized locomotion after 20 days of abstinence from a 20-day, daily cocaine injection regimen (Chapter 2), 2) blocked cocaine-primed reinstatement of extinguished cocaine-CPP (Chapter 2), and 3) blocked the expression, but not the acquisition of methamphetamine-CPP (Chapter 3). Using Fos as a marker of brain activity to study neural circuits involved in expression of methamphetamine-CPP and its attenuation by CP94253, I found that expression of methamphetamine-CPP was accompanied by increased Fos in the VTA and BlA, and decreased Fos in the CeA, however CP94253 pretreatment before the test reversed the conditioned changes in Fos expression in both amygdala subregions and enhanced levels of Fos in the VTA, NAcS, NAcC, and dmCPu (Chapter 3). Acute CP94253 in drug-naïve controls only increased Fos in the CeA, suggesting that the changes in Fos observed in experimental groups were not simply due to acute effects of CP94253 but rather were likely due to the agonist inhibition of CPP expression. Co-localization analyses revealed that approximately 70% of the Fos in the VTA and 50% of the Fos in the NAc co-localized with GAD67, whereas none of the Fos in the VTA co-localized with TH. These findings suggest that GABAergic cell activity, but not dopamine cell activity, in the VTA and NAc is likely involved in the behavioral effects of CP94253 on expression of methamphetamine conditioning. Collectively, these exciting

findings support 5-HT$_{1B}$Rs as a novel target for pharmacological intervention aimed at reducing the incentive motivation to seek methamphetamine.

The Role of 5-HT$_{1B}$Rs in Cocaine and Methamphetamine Addiction

Previous studies have found that pharmacological agonism of 5-HT$_{1B}$Rs increases cocaine intake during maintenance of self-administration (Parsons et al., 1998; Pentkowski et al., 2009; Pentkowski et al., 2014), but the same manipulation causes a decrease in cocaine intake and seeking after abstinence (Pentkowski et al., 2009; Pentkowski et al., 2014). The CP94253 attenuation of cocaine-CPP after extinction suggests that stimulation of 5-HT$_{1B}$Rs inhibits the incentive motivational effects of the cocaine-paired environmental cues that normally drive expression of CPP. Consistent with this interpretation, CP94253 attenuates cocaine self-administration on a progressive ratio schedule which requires increasing amounts of motivation and effort to obtain reinforcement (Pentkowski et al., 2014). Thus, responding under this type of schedule likely reflects incentive motivation to seek cocaine after abstinence or extinction.

The finding that CP94253 attenuated the expression of methamphetamine-CPP but had no effect on the acquisition of methamphetamine-CPP (Chapter 3) suggests that 5-HT$_{1B}$Rs are more critically involved in motivation to seek methamphetamine than in the unconditioned rewarding effects of methamphetamine. CP94253 did not block the acquisition of methamphetamine-CPP, which suggests that the rewarding value of methamphetamine and the learned association between methamphetamine and the environmental cues were not blocked by CP94253. However, the CP94253 attenuation of methamphetamine-CPP expression suggests that stimulation of 5-HT$_{1B}$Rs is needed for the incentive motivation for mice to seek the drug-paired chamber on the expression test

day. Collectively, our data are in line with previous work that has established a role of 5-HT_{1B}Rs in regulating both cocaine- and methamphetamine- seeking behavior (Fletcher & Korth, 1999; Garcia et al., 2017; Miszkiel et al., 2012; Pentkowski et al., 2009; Pentkowski et al., 2012; Pentkowski et al., 2014). However, 5-HT_{1B}R agonism attenuates conditioned reward and methamphetamine self-administration during maintenance (Fletcher & Korth, 1999; Garcia et al., 2017; Miszkiel et al., 2012) and self-administration post-abstinence (Garcia et al., 2017). It is possible that CP94253 not only differentially regulates drug abuse-related processes across different phases of the addiction cycle, but also has different effects on behavior depending on the specific addictive drug used, even those within the same drug-class. More research is needed to further investigate the role of 5-HT_{1B}Rs in reward.

The difference between 5-HT_{1B}R agonist effects on cocaine- and methamphetamine-induced behavior may be due to the properties unique to each of these stimulants. Both the amphetamines and cocaine inhibit 5-HT, dopamine, and norepinephrine transporters and cause a down-regulation of these transporters with repeated use (Azzaro & Rutledge, 1973; Ritz et al., 1990). However, they interact differently with the transporters. Cocaine inhibits monoamine transport back into the cell. In addition to inhibiting monoamine transport back into the cell, the class of amphetamine drugs redistribute intracellular monoamines by acting at the vesicular monoamine transporter (VMAT), which not only causes the release of monoamines into the cytosol but also reverses monoamine transport across the plasma membrane resulting in more monoamine release in the cytosol (Sulzer et al., 1995; Sager & Torres, 2011; Panenka et al., 2013). Additionally, cocaine and amphetamines produce differential

effects on the releasable vesicular pool and on regulation of VMAT-2 (Brown et al., 2001). Specifically, cocaine increases VMAT-2 activity while in contrast, methamphetamine reduces VMAT-2 function (Brown et al., 2001). This may result in a larger releasable pool of dopamine after cocaine versus methamphetamine following acute or subchronic administration. This may contribute to the differences seen with CP94253 during pre-abstinence testing between cocaine and methamphetamine.

The mechanisms underlying the effects of CP94253 on motivation to seek cocaine and methamphetamine are still unknown, however, the findings in this dissertation begin to unravel the neural circuitry involved in the agonist effects. In mice expressing methamphetamine-CPP, we observed an increase in Fos protein expression in the VTA and BlA, and a reduction in Fos protein in the CeA. However, when CP94253 blocked the expression of methamphetamine-CPP we found an increase in Fos protein expression in the VTA, NAcS, NAcC, and dmCPu, and importantly, CP94253 reversed the increased Fos observed in the BlA and the reduction seen in the CeA. The amygdala, VTA, and NAc are implicated in the rewarding effects of psychostimulants. We postulate that these circuitries are responsible for the inhibition of methamphetamine-CPP expression through attenuation of incentive motivation to seek the methamphetamine-conditioned environment. The VTA also receives projections from the dorsal CPu (Watabe-Uchida et al., 2012) and has been previously implicated in methamphetamine-CPP in rats (Liu et al., 2014), but not in mice (Chiang et al., 2009). Therefore, it is possible that the dmCPu also plays a role in the inhibition of methamphetamine-induced CPP expression following 5-HT_{1B}R agonism by CP94253.

Cell-specific markers co-labeled with Fos provided some insight into the circuitry

involved in CP94253 effects on methamphetamine-CPP expression. We observed that Fos protein expression occurred in GABA neurons and the proportion of GABA co-labeled cells depended on the brain region. In the VTA, approximately 70% of the Fos co-localized with GAD67 and no cells co-localized with TH, suggesting a role for GABA neurons and not DA neurons. It is possible that the remaining 30% of Fos expressing neurons were glutamatergic because glutamate neurons are also found in the VTA. It is surprising that our results suggest that the dopaminergic VTA → NAc, the hallmark mesolimbic pathway involved in drug addiction, did not exhibit Fos expression but rather Fos expression was driven primarily by the VTA GABAergic neuronal population. In the NAc, approximately 50% of the Fos labeled cells were GAD67-expressing neurons, indicating a role for GABA neurons in the NAc as well. The remaining 50% of Fos labeled cells in the NAc could potentially be cholinergic interneurons, another neuronal family found in the NAc (Berlanga et al., 2003; Witten et al., 2010) or glutamate neurons (Di Ciano & Everitt, 2001).

My interest in the effects 5-HT_{1B}R agonists on methamphetamine CPP was in part due to previous work from our lab demonstrating that the FDA approved 5-$TH_{1B/1D}$R agonist, zolmitriptan, alters methamphetamine abuse-related behaviors (Garcia et al., 2017). Garcia et al. found that zolmitriptan, given acutely during maintenance of methamphetamine self-administration attenuated intake on a VR5 reinforcement schedule. When administered intermittently (every 2-3 days) following abstinence, zolmitriptan also decreased methamphetamine intake, suggesting that this 5-$HT_{1B/1D/1A}$R agonist can reduce the reinforcing properties of methamphetamine. My findings suggest further investigating the therapeutic potential of FDA approved 5-HT_{1B}R agonists for the

treatments of SUDs.

Future Directions

Our findings are exciting as they identify the role serotonin acting at 5-HT_{1B}Rs plays in regulating the effects of both cocaine and methamphetamine. Future work is needed to explore the mechanism by which 5-HT_{1B}Rs produce their inhibitory effects on psychostimulant abuse-related behavior, as well as the generalizability of our effects to other drug classes. Nearly every drug of abuse increases dopamine levels in the NAc (Hyman et al., 2006), but the mechanism differs across drug classes. Given these differences and the commonality of polydrug abuse, future work aimed at testing the effects of CP94253 on other drugs of abuse, such as heroin or morphine, is warranted.

Other important questions that remain are whether effects of 5-HT_{1B}R agonist depend on age, sex, character traits such as impulsivity and compulsivity, or comorbidity with other mental illness. Indeed, 5-HT plays an essential role in various brain functions including feeding, sleep, pain, mood, aggression, impulsivity, thermoregulation, locomotion, and learning, and has been implicated in a wide range of other psychiatric conditions including depression, anxiety disorders, obsessive–compulsive disorder, psychosis, and eating disorders (for review, see Lucki, 1998). Therefore, it seems likely that there are individual differences in sensitivity and effects of 5-HT_{1B}R agonists on drug abuse-related behaviors that may be discovered in future research.

Sex differences in the effects of drugs of abuse exist both in animal studies and in a clinical setting and should be considered while conducting drug abuse research (Brady & Randall 1999; Carroll & Anker, 2010; Hankoskye al., 2018). Additionally, male and female rodents respond differentially when it comes to chronic stress, which is a known

predictor of drug abuse (Enoch, 2011). Female rodents tend to exhibit higher motivation for drugs across multiple phases of dependence (Becker & Hu, 2008; Lynch & Carroll, 1999; for review, see Carroll & Anker, 2010). Even though we did not examine the effects of our manipulation in female mice in the current study, a current ongoing study in our laboratory is investigating the effect of the 5-HT_{1B}R agonist CP94253 in female rats using a self-administration paradigm (Scott et al., manuscript under preparation). Results thus far suggest that CP94254 shifts the dose effect function for cocaine intake during the maintenance phase of cocaine self-administration to the left, similar to our previous findings in male rats (Pentkowski et al., 2009; Pentkowski et al., 2014). After a 21-day abstinence phase, CP94253 reduces cocaine intake just as it does in male rats, regardless of estrus cycle phase. These results provide promising evidence that females respond similarly to CP94253 as males, and therefore, may also benefit from agonist treatment post abstinence. Further studies in females examining methamphetamine addiction-related behaviors are warranted.

One limitation of this dissertation research is that we did not demonstrate that the effects are reversed by a $5HT_{1B}$R antagonist to definitively demonstrate the effects were 5-HT_{1B}R-mediated. However, previous researcher from our lab has demonstrated that 5-HT_{1B}R antagonist administration reverses both the attenuation and enhancement produced by CP94253 in rats self-administering cocaine or methamphetamine (Pentkowski et al., 2014; Garcia et al., 2017). CP94253 is a selective 5-HT_{1B}R agonist with approximately 25- and 40-fold more selectivity for 5-HT_{1B}Rs over 5-HT_{1D} and 5-HT_{1A}Rs, respectively (K_i values are 89, 2, 49 and 1,600 nM for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, and 5-HT_2Rs respectively; Koe et al., 1992). It is important to note that since CP94253

also has affinity for 5-HT_{1D}Rs (K_i = 49 nM; Koe et al., 1992) it is possible that 5-HT_{1D}Rs may also contribute to its effects on cocaine- and methamphetamine- seeking behavior. However, recent data using a 5-HT_{1D}R agonist failed to block the expression of methamphetamine-CPP in rats elucidating a primary role for the 5-HT_{1B}Rs (Shahidi, Komaki, Sadeghian, & Soleimani, 2018). More research is needed to determine the potential contribution of other receptors from the serotonin 1 family to the effects observed with CP94253 in the present studies.

We used mice for this dissertation research with the idea that the tools for genetic manipulation are much more available and advanced than those for rats. However, with the advantages come disadvantages, one being the integrity of surgically implanted cannula in mice that are group housed for these extended experiments, and another being the differences in stress response between species (Hoplight et al., 2007; Ryabinin et al., 1999;). Daily repeated injections across days causes chronic stress in mice even though it is only a mild stressor for rats. We observed clear cross-sensitization between cocaine and injection stress, and the results further suggested that group housing drug naïve mice with methamphetamine-treated mice was also a stressor that cross-sensitizes with methamphetamine (Chapter 2). We were able to decipher effects of the chronic stress in the saline control group in this study by changing our housing conditions. However, in our cocaine group, it is difficult to separate the effects of cocaine from chronic injection stress which is inherent in the cocaine administration regimen. It would be beneficial to assess chronic cocaine effects separately from chronic cocaine + stress effects but that is challenging in mice. Given that a contributor to relapse and initial drug use is stress, it would be interesting to parse out how much of a contributor stress is to continued use of

an illicit substance. Although stress was a limitation for aspects of the current research, chronic stress in studies concerning mental health outcomes may provide an even more accurate model.

Mental illness tends to be comorbid with SUDs and vice versa (Kelly & Daley, 2013; Ross & Peselow, 2012). Around 1 in 4 individuals with a serious mental health illness also have some type of co-occurring SUD, although this does not necessarily mean that one caused the other (www.drugabuse.gov/publications/research-reports/common-comorbidities-substance-use-disorders). High rates of comorbidity are seen with anxiety disorders, as well as depression (Conway, Compton, Stinson, & Grant 2006; Torrens, Gilchrist, & Domingo-Salvany, 2011). SUDs have a high prevalence with psychotic illness, ADHD, bipolar, and personality disorders (De Alwis, Lynskey, Reiersen, & Agrawal, 2014; Florez-Salamanca et al., 2013; Torrens et al., 2011). Preclinical studies suggest that 5-HT_{1B}Rs may play a role in such comorbidities. For instance, Nautiyal et al. (2016) found that blocking 5-HT_{1B}R autoreceptors using an inducible knock-out mouse attenuates behaviors that model anxiety and depression. Often those with mental illness 'self-medicate' with substances. Drug use can also enhance or bring about symptoms of mental illness such as psychosis. Thus, it is important to consider if 5-HT_{1B}R functionality and expression is affected by mental illnesses, and if 5-HT_{1B}R regulation of cocaine- and methamphetamine- addictive behaviors is changed in a comorbid model.

Impulsivity is a known predictor of SUDs especially in combination with other factor such as stress, anxiety, and genetic phenotype. Impulsivity is a complex trait involving 1) an inability to reflect on the consequences of ones' actions; 2) an inability to forego an immediate smaller reward for a larger reward in the future; and/or 3) a deficit

in suppressing prepotent motor responses (Chamberlain & Sahakian, 2007). For example, high impulsivity precedes the escalation of cocaine self-administration behavior and the tendency toward compulsive cocaine-seeking and relapse (for review, see Dalley, Everitt, & Robbins, 2011). Furthermore, research has shown the maturation of connections between the PFC, basal ganglia, and cerebellum are crucial for the development of higher cognitive functions (Delgado 2007; Hare et al., 2008; Heyder, Suchan, & Daum, 2004). Those who are more impulsive tend to be risk-takers and have reduced control of their impulses. In a preclinical analogue, Nautiyal et al. (2015) found that expression of 5-HT_{1B}R heteroreceptors in adulthood modulates impulsive behavior using an inducible knock out mouse model. It would be interesting to replicate some of our current findings using a strain of impulsive mice and investigate possible alterations in the role the 5-HT systems play in addiction within this strain.

Drug use is often initiated during early adolescence (Bukstein & Horner, 2010; Kandel & Logan, 1984; Sheidow, McCart, Zajac, & Davis, 2012) and typically when the first signs of mental illness appear. Peri-adolescent rodents tend to be more sensitive to the rewarding and reinforcing effects of drugs of abuse (O'Dell, 2009). This distinguishing feature makes adolescent rodents and humans more vulnerable to drug effects than adults. Executive control circuits such as decision making and inhibition of impulses, as well as frontolimbic circuits continue to develop until early adulthood and are among the last group of circuits to mature (Hare et al., 2008; Kelly & Daley 2012; Winters et al., 2014). Neurobiological differences in the brain reward circuitry exist between adolescents and adults (for review, see Schepis, Adinoff, & Rao, 2008) including differences in basal dopamine levels (Stansfield & Kirstein, 2005), receptor

pruning (Seeman et al., 1987), and differences in maturation in the cannabinoid, glutaminergic, GABAergic and 5-HTergic systems (for review, see Schepis et al., 2008). The PFC circuitry not being fully development leaves adolescents with less inhibition of executive function and impulse control along with an increase in risk-taking behavior (for review, see Schepis et al., 2008). Previous research does show that adolescent rodents respond to serveal drugs, including cocaine, methamphetamine, alcohol, differently than adults (for review, see Spear 2016). Given the lack of fully developed circuits during the adolescent age period (for review, see Schepis et al., 2008), it would be noteworthy to test the effects of 5-HT_{1B}R agonists on cocaine and methamphetamine use initiated during early adolescence. Peri-adolescence in mice is approximately postnatal day (PND) 33 and the current experiment's used mice between PND 45-50 at the beginning of the experiment. It would be interesting to determine if the enhancement and attenuation of drug-seeking behaviors follow patterns similar to that of adult rodents. Given that drug abuse often begins in adolescence, which is a critical period of vulnerability given immature circuitry, the effects of CP94253 can be tested with initial drug exposure initiating during the early developmental stage.

Conclusion

This dissertation supports the hypothesis that the 5-HT_{1B}R agonist CP94253 can attenuate cocaine- and methamphetamine-seeking as well and non-conditioned drug-related behaviors, albeit during different stages of the addiction cycle. The data from these experiments suggest that 5-HT_{1B}Rs reduce the motivation for cocaine and methamphetamine. Potential mechanisms underlying these effects may include the amygdala, NAc, and VTA neurocircuitries. Future lines of research should further

explore neuronal subtype specificity as well as the FDA approved 5-HT1B/1DR agonist Zomaltriptan. Lastly, it is important to investigate if the rodent model translates to cessation of drug seeking in humans. The findings from this dissertation point to an exciting avenue in understanding the influence of 5-HT_{1B}Rs in the development, expression, relapse, and treatment for SUDs.

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APPENDIX A

TABLES

Table 1

Percent of Fos Co-localization with GAD67 in Methamphetamine-Conditioned mice and Saline-Controls

Brain Region	N	% Co-localization Fos+GAD67
VTA	26	69.7%
dmCPu	26	55.4%
NAcC	22	57.9%
NacS	22	45.9%
CeA	28	63.2%
BlA	27	20.2%
PrL	27	67.0%
IL	26	34.4%

Note. Mean+SEM of Fos positive cells co-localize with GAD67 per mm^2 for the VTA, dmCPu, NAcS, NAcC, CeA, BlA, PrL, and IL. Mice were conditioned with saline or methamphetamine (1 mg/kg, IP) for 4 consecutive days. Mice that formed a methamphetamine-CPP were divided into 2 groups for the expression test which occurred 72 hours after the CPP test. Saline controls received saline at all time points. One group of mice conditioned with methamphetamine was pretreated with saline and the other with 10 mg/kg CP94253 30 min prior to the 15-min Expression test. 75 minutes after the behavior test mice were perfused transcardially and brains were harvested. Tissue was sliced at 40 microns. We determined the density of Fos and GAD67+Fos, per mm^2 of each analyzed region. To calculate the percent of Fos cells co-labeled with GAD67, we divided number of Fos cells co-labelled with GAD67 by total Fos labelled cells.

APPENDIX B

FIGURES

A

EXPERIMENT 1 TIMELIME			
Days 1-20	**Day 21**	**Days 22-41**	**Day 42**
Chronic daily injections	**Pre-abstinence test**	**Abstinence (i.e., no injections)**	**Post-abstinence test**
Pretreatment (saline or 10 mg/kg CP94253)	**30 min →**	**Challenge (saline or 5 mg/kg cocaine)**	

B

EXPERIMENT 3 TIMELINE					
Days 1-11	**Days 12-13**		**Day 14-19**	**Day 20**	**Day 21**
Daily injections	**Habituation/BL test**		**Conditioning**	**Rest day**	**CPP test**
	Daily injections continue				
Days 22-34	**Day 35**	**Day 36**	**Days 37-40**	**Day 41**	**Day 42**
Extinction	**Extinction test***	**Reinstatement test**			
***Mice needing additional extinction training**		**Rest day**	**Extinction**	**Extinction test**	**Reinstatement test**

Figure 1. Time for cocaine locomotor experiment **(A)**. Timeline for cocaine CPP extinction-reinstatement experiment **(B)**.

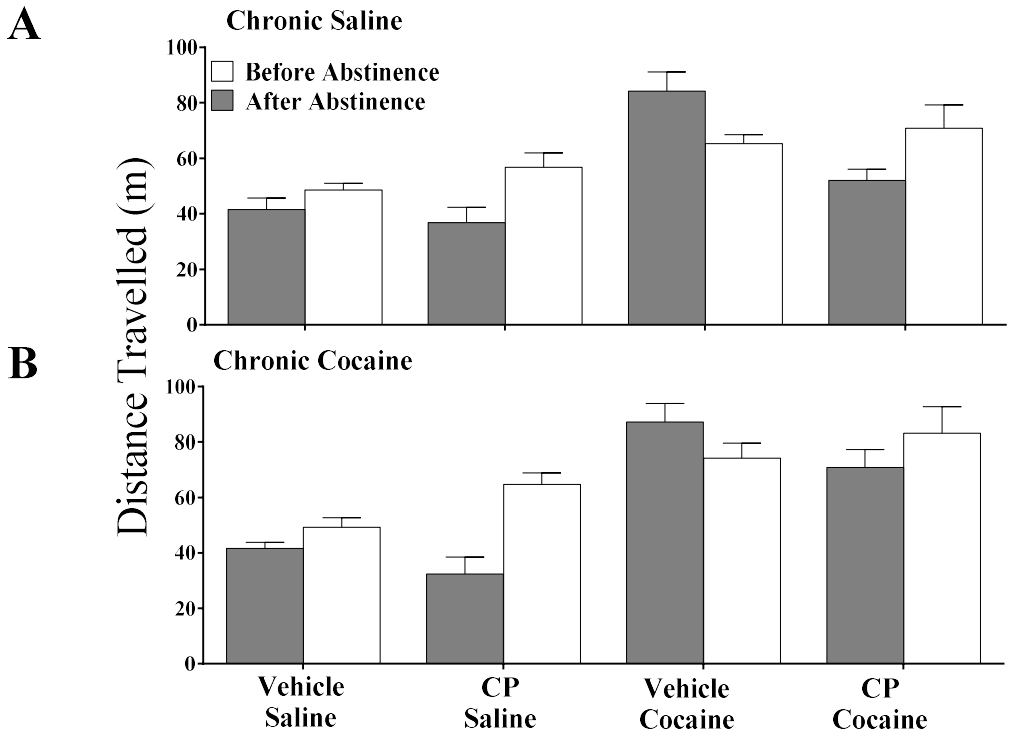

Figure 2. Distance traveled (meters ± SEM) by mice that received either chronic daily injections of saline **(A)** or 10 mg/kg cocaine **(B)** and were tested both 24 h after the last of 20 injections (i.e., before abstinence, white bars) and 20 days after (i.e., after abstinence, gray bars), n=8-11/group. Contrary to prediction, there was no effect of chronic treatment conditions nor interactions with cocaine challenge (0 or 5 mg/kg, IP) or CP94253 (0 or 10 mg/kg, IP), so further analyses were conducted averaged across the chronic treatment variable.

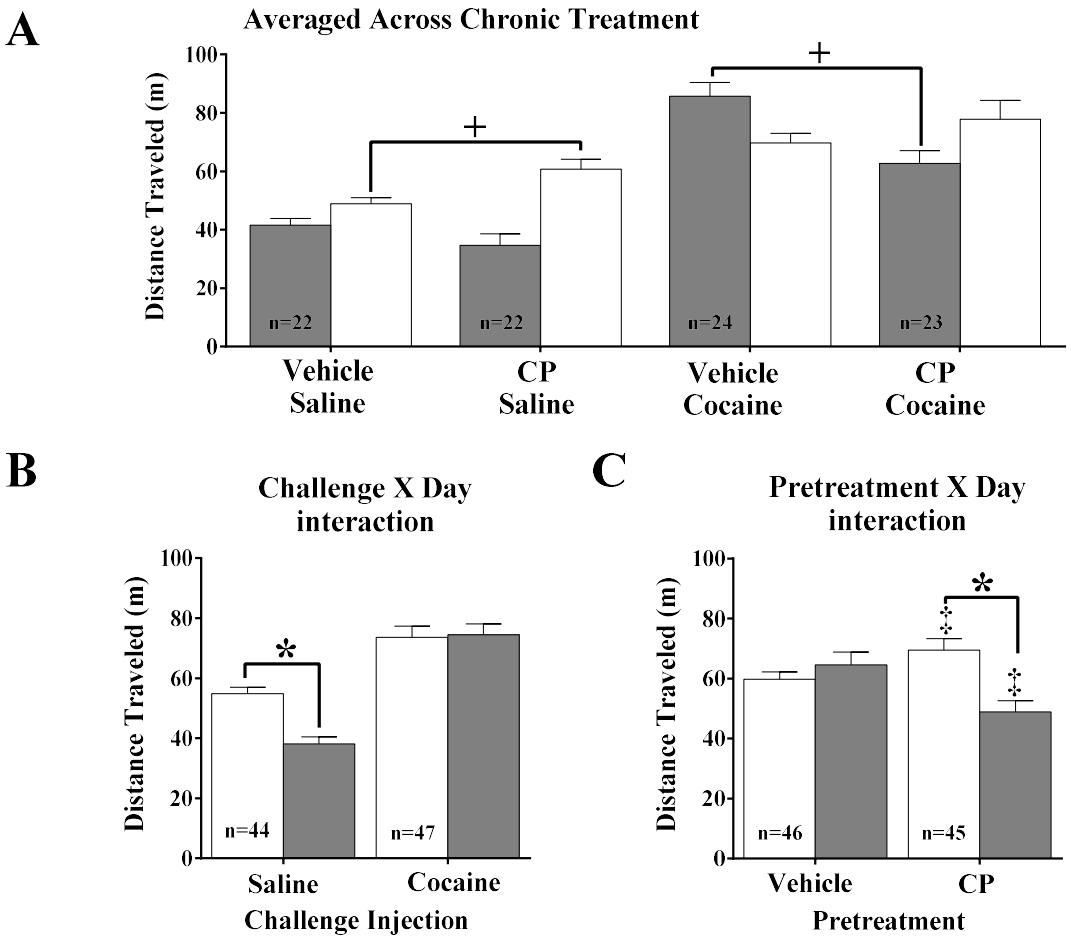

Figure 3. Contrary to prediction, there was no effect of chronic treatment conditions nor interactions with cocaine challenge (0 or 5 mg/kg, IP) or CP94253 (0 or 10 mg/kg, IP), so further analyses were conducted averaged across the chronic treatment variable **(A)**. This analysis yielded a challenge injection by day interaction **(B)** and a pretreatment by day interaction **(C)**. Asterisk (*) represents a significant post-hoc comparison, $p<0.05$; Plus sign (+) represents a significant planned comparison, $p<0.05$. Double plus (‡) represents a significant difference from respective vehicle condition, Bonferroni t-test $p<0.001$.

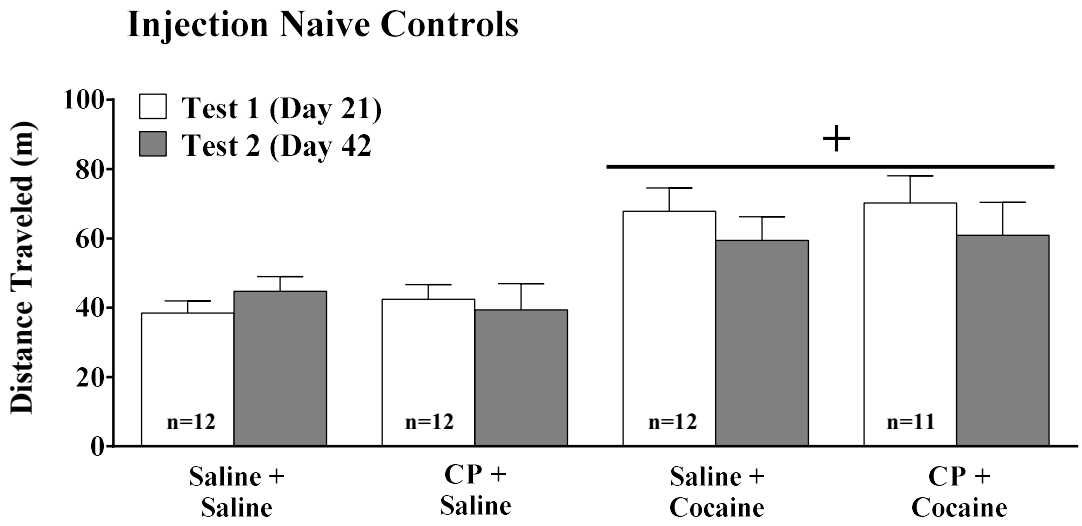

Figure 4. Distance traveled (meters ± SEM) by injection-naïve mice that were treated the same as mice in the previous experiment (see timeline on Figure 1) except that they were not given daily injections over the first 20 days of the experiment, but were instead left undisturbed in their home cages except for twice weekly tail marking for identification. On the test days, the mice received an injection of either saline or CP94253 (10 mg/kg, IP) and 30 min later received a saline or cocaine (5 mg/kg, IP) injection (n=11-12/group). Plus sign (+) represents a significant difference from saline-challenged groups, $p<0.001$

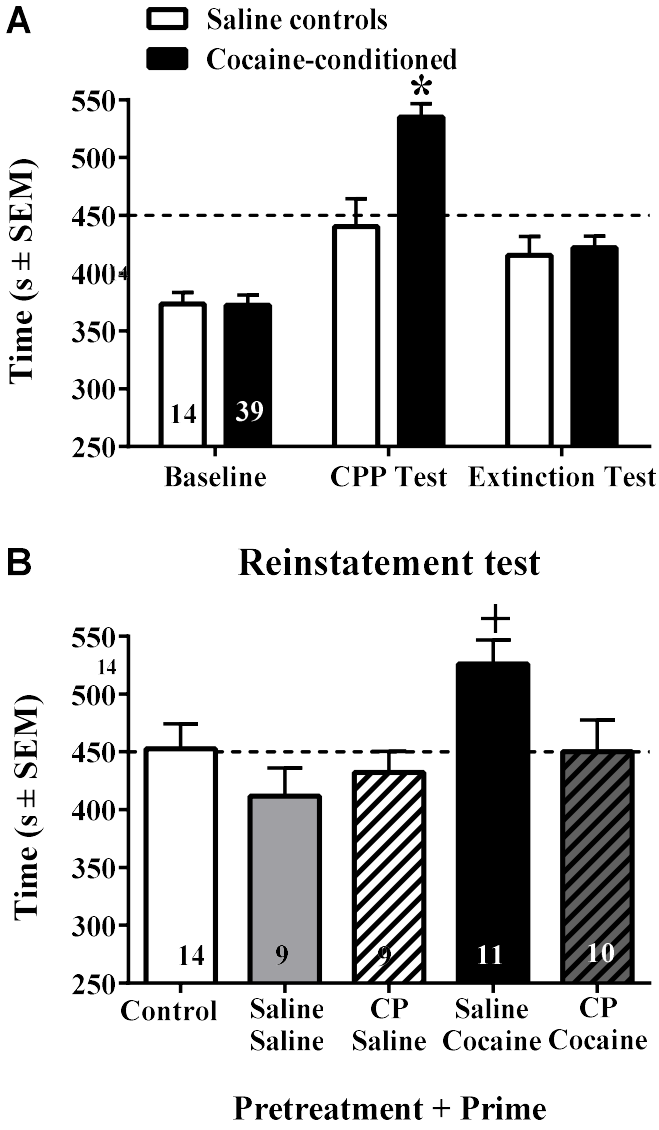

Figure 5. Results of 15-min preference tests to assess baseline preference, cocaine-CPP, and extinction of cocaine-CPP **(A)**. Subsequently, mice that had received repeated saline injections (white bars) or repeated cocaine injections (black bars) prior to and during conditioning were tested for reinstatement of CPP **(B)** following pretreatment with either saline or CP94253 (10 mg/kg, IP) and a priming injection of either saline (Sal) or cocaine (15 mg/kg, IP; Coc) 30 min later and immediately prior to the test (n=9-11/group). Values are the time (s ± SEM) in the initially non-preferred compartment (i.e., cocaine-paired side for conditioned mice) and dashed line represents 50% of the total test time such that values above the line illustrate a preference switch. Asterisk (*) represents difference from saline group, Bonferroni t-test $p<0.001$. Plus (+) represents difference from all other groups, Tukey test, $p<0.05$.

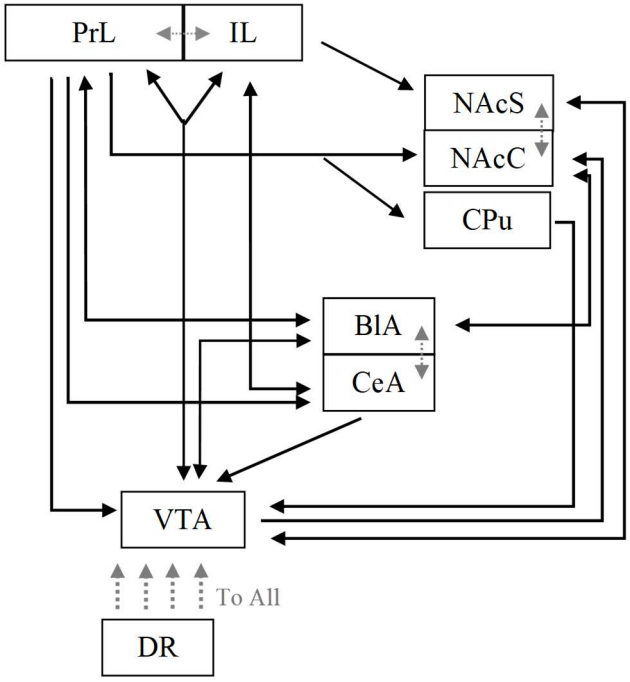

Figure 6. Schematic representing efferent and afferent connections of the prelimbic cortex (PrL), infralimbic cortex (IL), nucleus accumbens shell (NAcS) and core (NAcC), caudate-putamen (CPu), basolateral amygdala (BlA), central nucleus of the amygdala (CeA), ventral tegmental area (VTA), dorsal raphe nucleus (DRN).

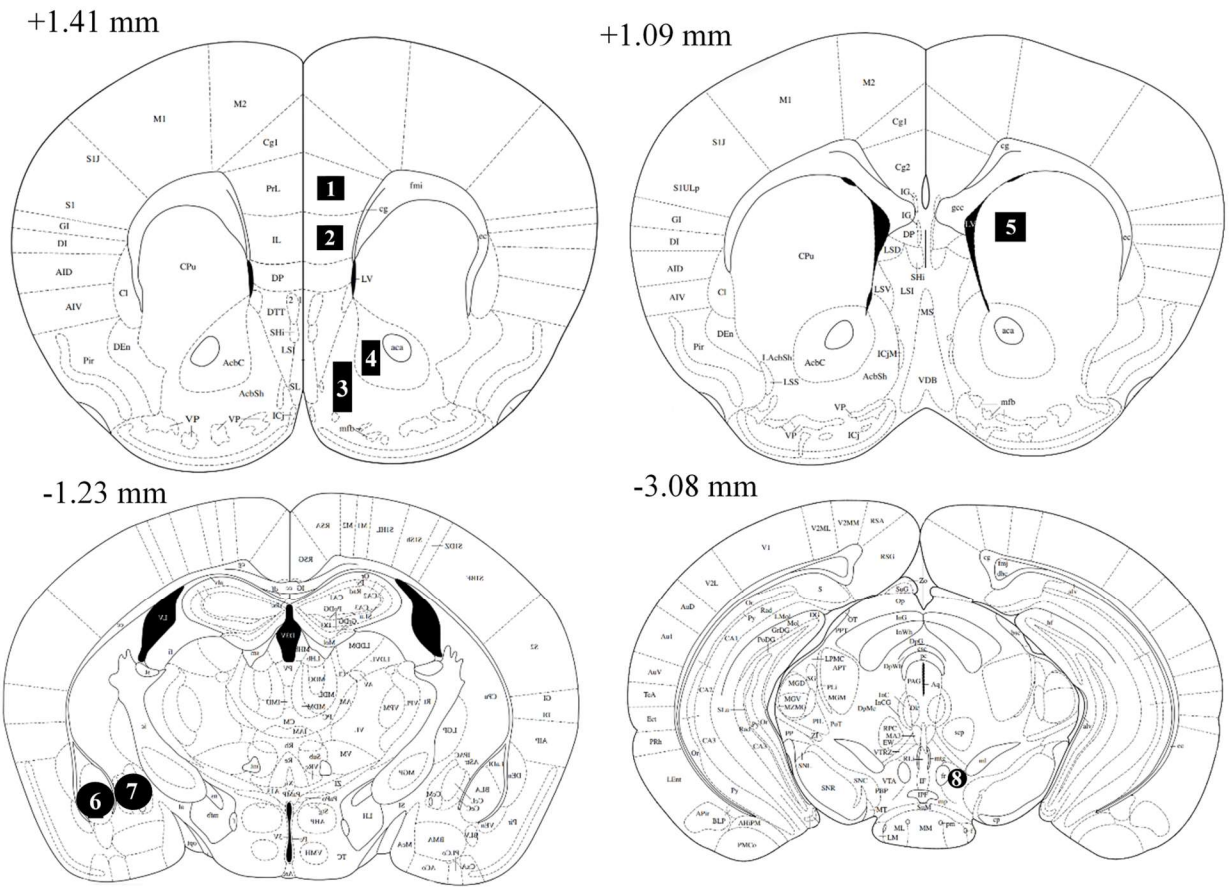

Figure 7. Schematic representation of coronal sections of the mouse brain taken at +1.41, +1.09, -1.23, and -3.07 mm from Bregma (Paxinos and Franklin, 2013). Numbers in the sections represent the regions analyzed for Fos as follows: (1) Prelimbic region (PrL) of the medial prefrontal cortex (mPFC); (2) Infralimbic region (IL) of the mPFC; (3) nucleus accumbens core (NAcC); (4) nucleus accumbens shell (NAcS); (5) dorsal medial caudate-putamen (dmCPu); (6) basolateral amygdala (BlA); (7) central nucleus of the amygdala (CeA); (8) ventral tegmental area (VTA).

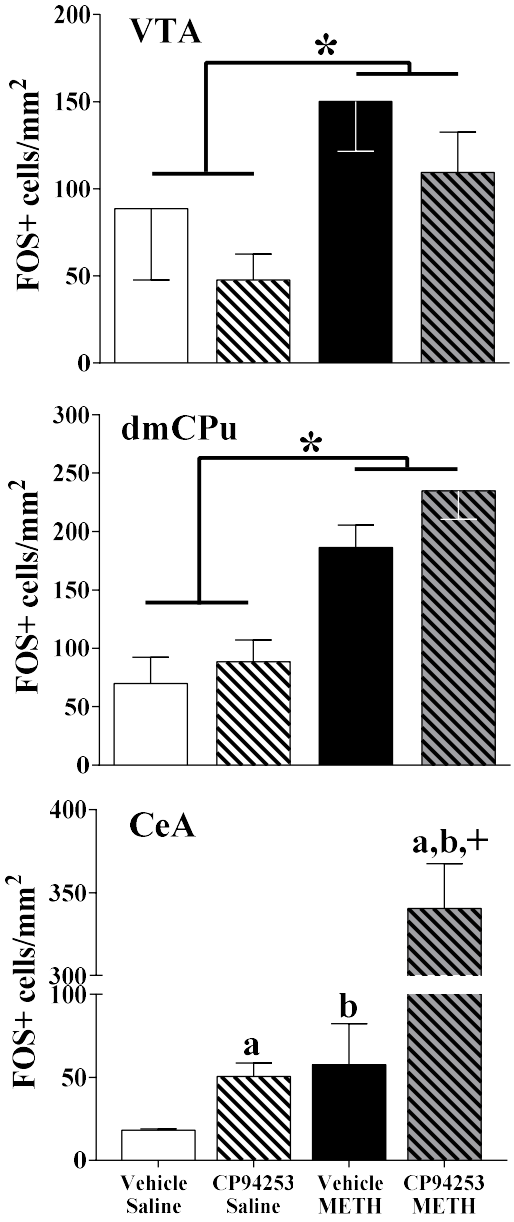

Figure 8. Mean+SEM of Fos-positive cells per mm^2 measured in the VTA (top), dmCPu (middle), and CeA (bottom) in mice pretreated with vehicle or CP94253 (10 mg/kg, IP) 30 min prior to a second injection of either saline or methamphetamine (METH; 3 mg/kg, IP; n=3-5/group). Brains were harvested 90 minutes after the second injection. Asterisk (*) represents ANOVA main effect of treatment (methamphetamine), $p<0.05$; (a) represents ANOVA main effect of Pretreatment (CP94253) $p<0.001$; (b) represents ANOVA main effect of treatment (methamphetamine), $p<0.001$; (+) represents difference from all other groups, Bonferroni t-test, $p<0.001$.

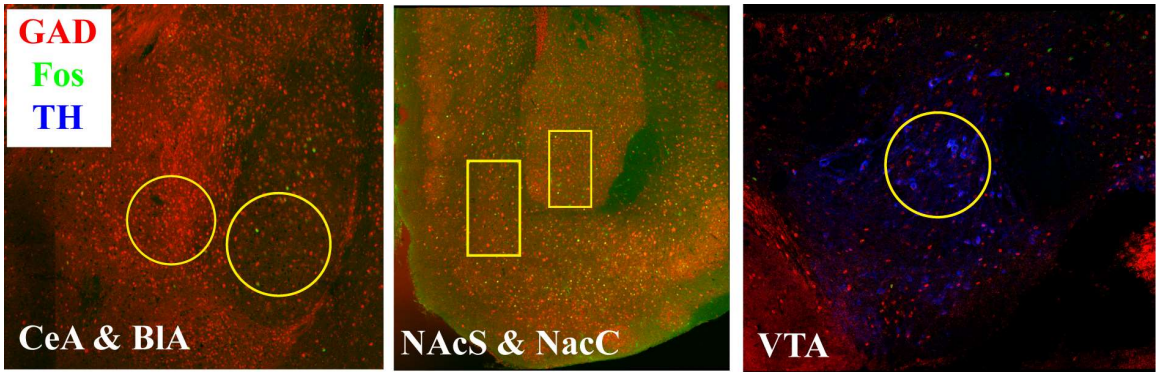

Figure 9. Representative photomicrographs of the CeA, BlA, NacS, NacC, and VTA analyzed for Fos in all immunofluorescence experiments. Fos (Green), GAD67 (Red), and TH (Blue). GAD67 is a marker for GABA neurons while TH is a marker for dopaminergic.

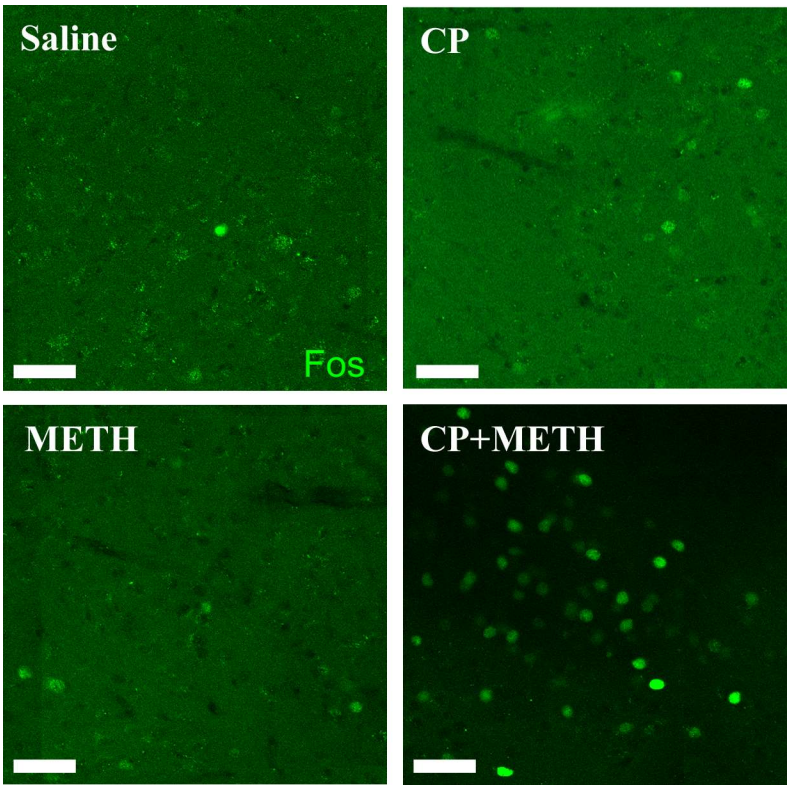

Figure 10. Unconditioned effects of CP94253 (10 mg/kg, IP) and methamphetamine (METH; 3 mg/kg, IP) on Fos Expression (Green) in the CeA. Representative photomicrographs from Experiment 4 showing coronal sections at 20× magnification of the CeA showing the acute effects of CP94253 (10 mg/kg, IP). There is increased Fos in mice treated with either CP92453 or methamphetamine. When CP94253 was administered in combination with methamphetamine Fos increased compared to all other groups, Bonferroni t-test, $p<0.001$. Scale Bar = 50 microns.

A

Timeline for acquisition of methamphetamine CPP (Experiment 5)					
Days 1-3	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**
Habituation and Baseline tests	**Drug pairing**	**Saline pairing**	**Saline pairing**	**Drug pairing**	**CPP test**

B

Timeline of expression of Meth-CPP experiment (Experiment 6)			
Days 1-3	**Days 4-7**	**Days 9-10**	**Day 11**
Habituation Baseline tests	**Saline pairing AM session Meth pairing PM session**	**OFF**	**Expression test**

Figure 11. Timeline of Experiment 5 examining the acquisition of methamphetamine-CPP (n=8-12/group). and Experiment 6 examining the expression of methamphetamine CPP (n=10-12/group).

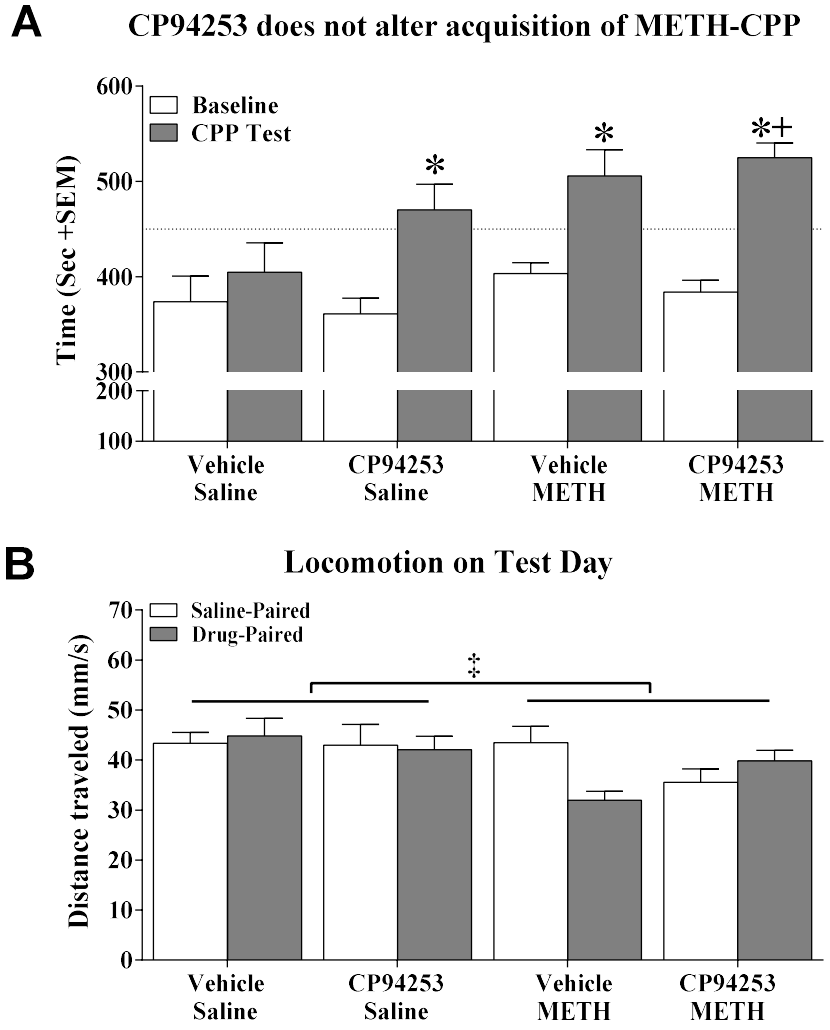

Figure 12. The time (sec +SEM) mice spent in the initially nonpreferred side during 15-min preference tests to assess baseline preference (BL; white bars) and acquisition of methamphetamine-CPP (CPP; gray bars) are shown in A and locomotor activity rate (mm traveled/sec + SEM) is shown in B (n=8-12/group). The group titles on the X-axis indicate the animals' pretreatment with either vehicle or CP94253 (10 mg/kg, IP) 30 min prior to receiving either saline or methamphetamine (METH; 3 mg/kg, IP). Mice were placed into their initially nonpreferred side immediately after these injections and during alternate sessions (see timeline) all groups received vehicle followed by saline 30 min later and were placed immediately into their initially preferred side. Dashed line represents 50% of the total test time such that values above the line illustrate a preference switch. Asterisk (*) represents difference from baseline, t-tests with Bonferroni correction, $p<0.01$. Plus (+) represents difference from vehicle/saline group, t-tests with Bonferroni correction, $p<0.01$. Double plus (‡) represents main effect of conditioning treatment $p<0.01$.

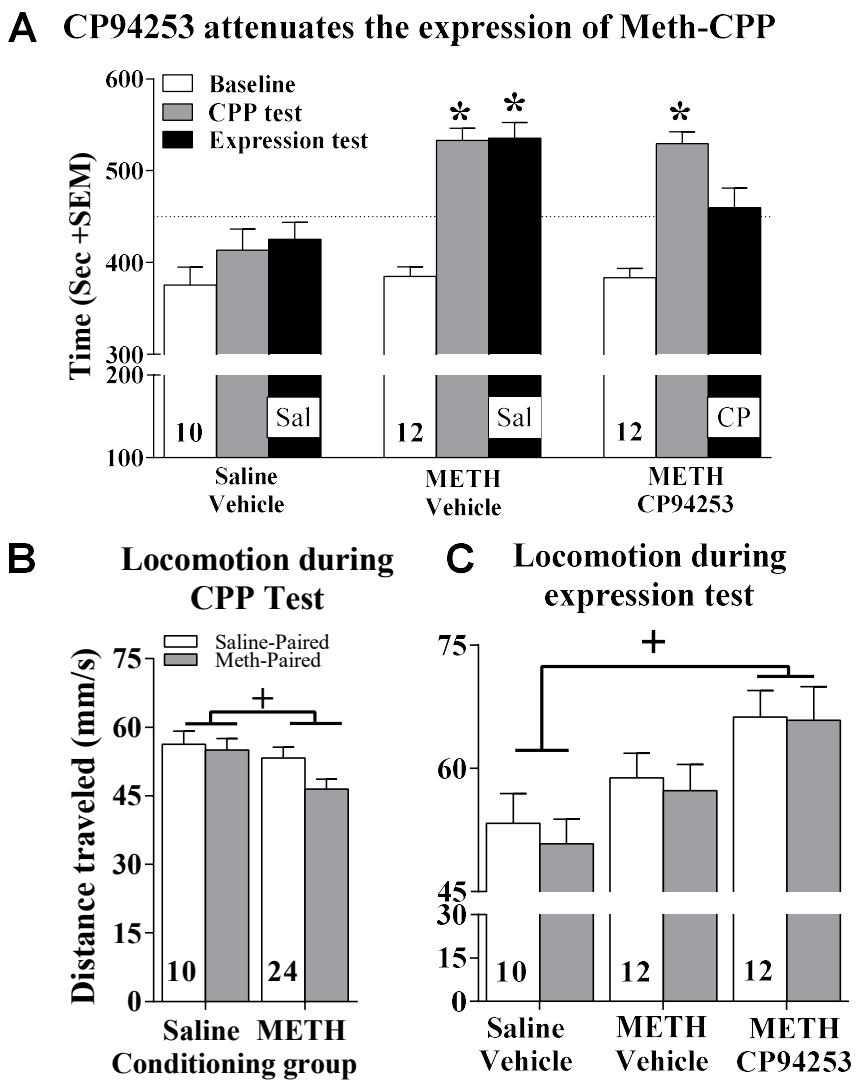

Figure 13. The time (sec +SEM) mice spent in the initially nonpreferred side during the 15-min preference tests to assess baseline preference (white bars), establishment of methamphetamine-CPP (CPP test; gray bars), and expression of methamphetamine-CPP (Expression test; black bars) is shown in A. The group titles on the X-axis indicate the drug that mice received immediately prior to placement into the initially nonpreferred side during conditioning [i.e., either saline or 1 mg/kg, IP methamphetamine (METH)] and the drug that they received 30 min prior to the expression test (i.e., either vehicle or 10 mg/kg, IP CP94253). All mice received saline immediately prior to placement into the initially preferred side during conditioning and no pretreatments were given prior to baseline or the initial CPP test. Dashed line represents 50% of the total test time such that values above the line illustrate a preference switch. Rate of distance travelled (mm/second +SEM) is shown for the initial CPP test day **(B)** and the Expression test day **(C)**. Asterisk (*) represents difference from baseline, t-tests with Bonferroni correction, $p<0.01$. Plus (+) represents main effects, $p<0.05$.

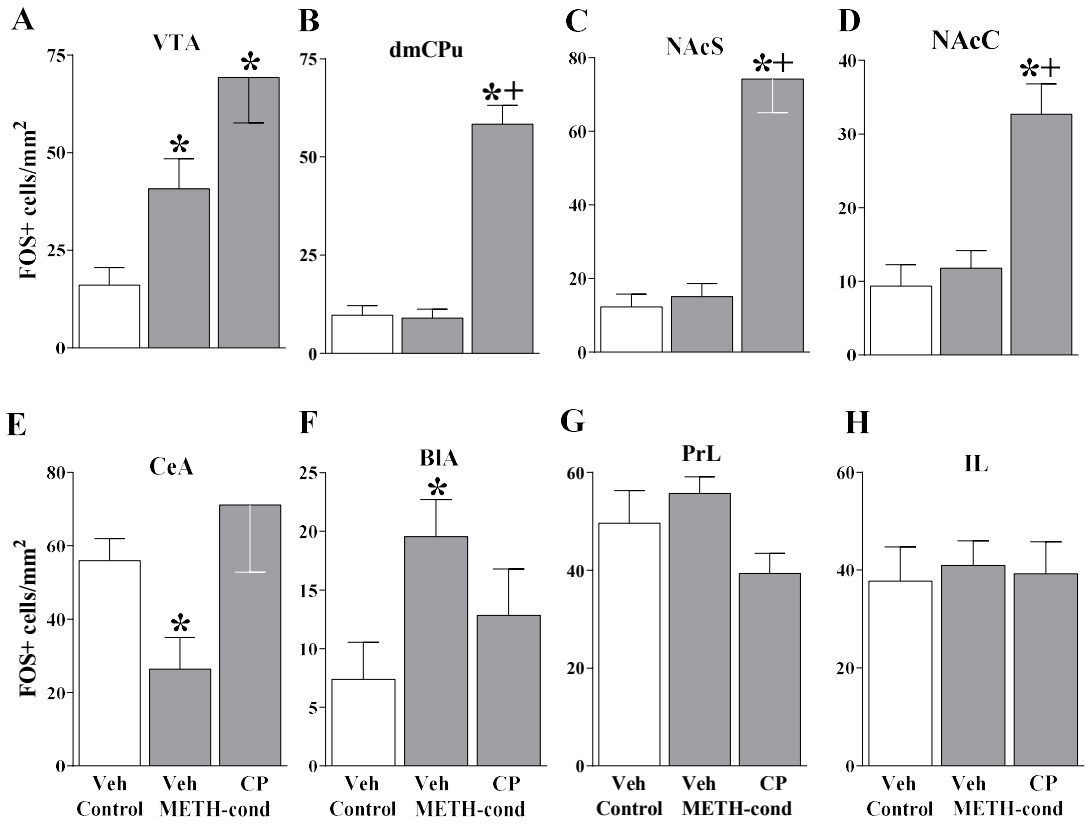

Figure 14. Mean+SEM of Fos positive cells per mm^2 for the (A) VTA, (B) dmCPu, (C) NAcS, (D) NAcC, (E) CeA, (F) BlA, (G) PrL, and the (H) IL (n=7-10/group). Mice were conditioned with saline or methamphetamine (1 mg/kg, IP) for 4 consecutive days. Mice that formed a methamphetamine-CPP were divided into 2 groups for the expression test which occurred 72 hours after the CPP test. Saline controls received saline at all time points (white bars). One group of mice conditioned with methamphetamine was pretreated with saline (middle grey bars) and the other with 10 mg/kg CP94253 (rightmost grey bars) 30 min prior to the 15-min Expression test. 75 minutes after the test (i.e., 120 min post-pretreatment), mice were perfused transcardially, brains were harvested, and tissue slices (40 microns) were labeled for Fos. Asterisk (*) represents difference from control group, t-tests with Bonferroni correction $p<0.016$. Plus (+) represents difference from vehicle (Veh) pretreated methamphetamine-conditioned (METH-cond) group, t-tests with Bonferroni correction, $p<0.016$.

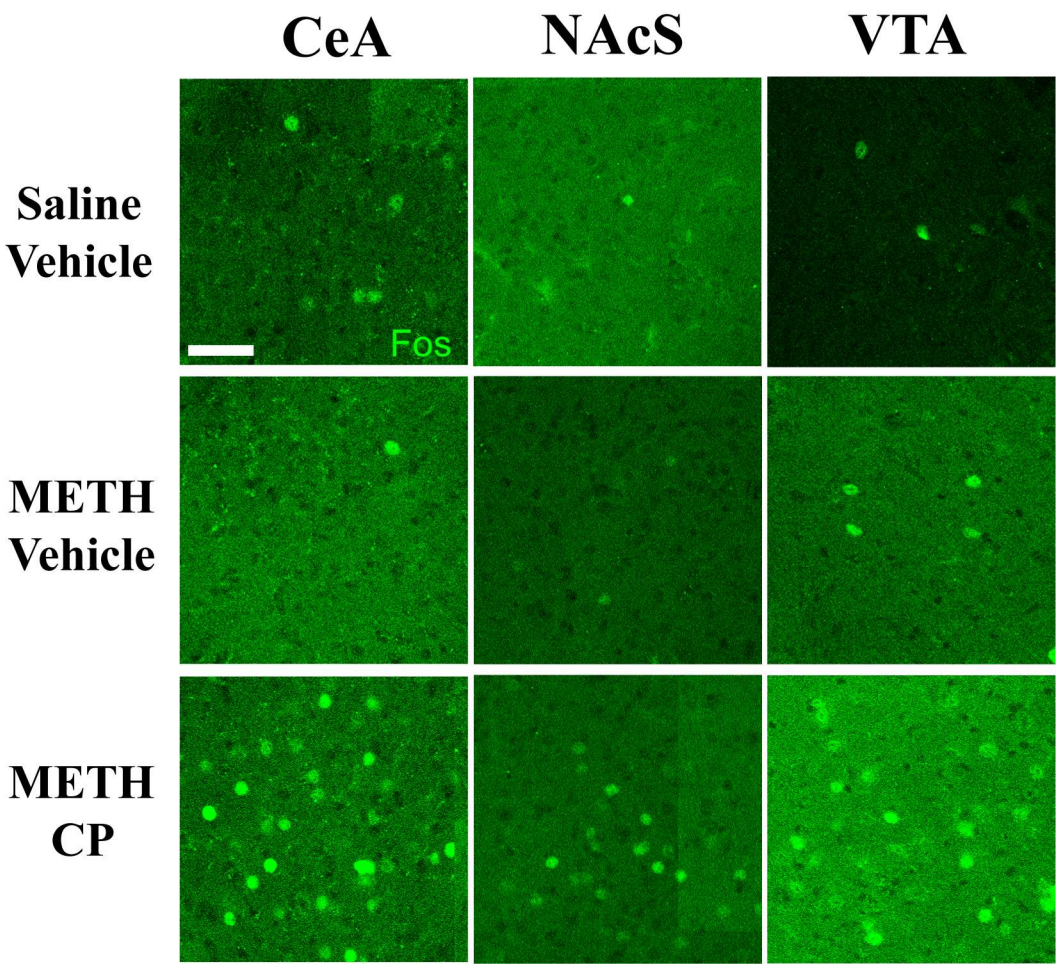

Figure 15. Representative photomicrographs from Experiment 6 showing coronal sections at 20× magnification in the CeA (left column), NAcS (middle column), and VTA (right column). Effects of CP94253 (10 mg/kg, IP) on Fos Expression (Green) in methamphetamine-conditioned (METH; 1 mg/kg, IP) and saline behavioral control mice. Methamphetamine-conditioned mice expressed reduced Fos in the CeA and increased Fos in the VTA. CP94253 increased Fos in the NAcS and VTA and reversed the decrease observed in the CeA. Scale bar = 50 microns.

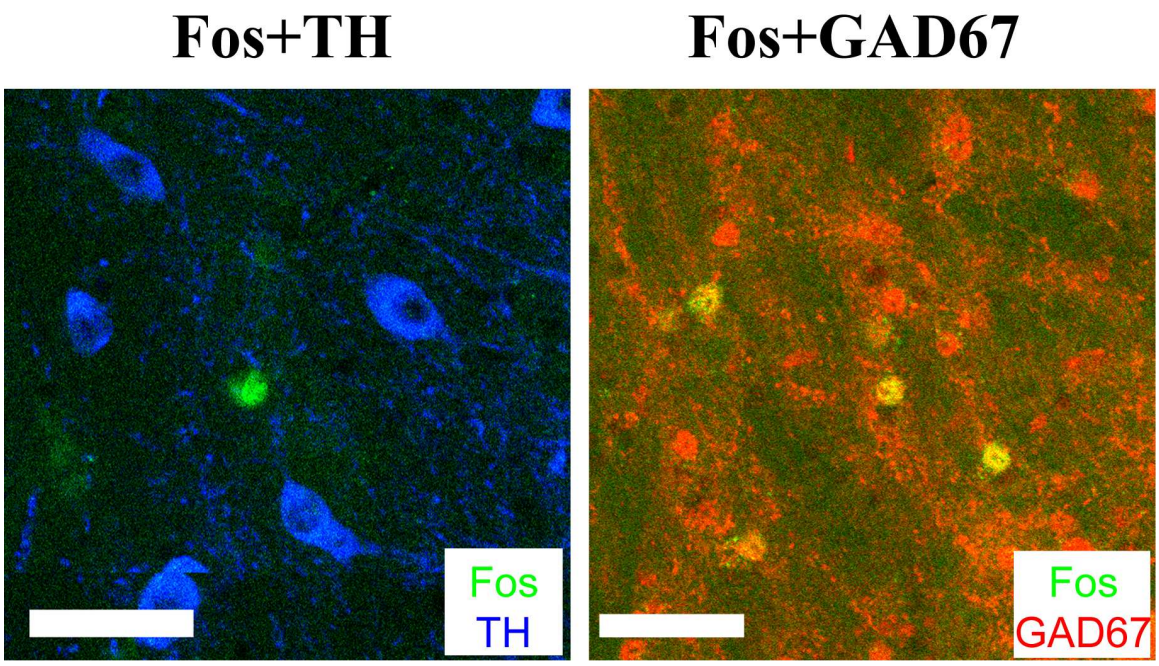

Figure 16. Representative photomicrographs from Experiment 6 showing coronal sections at 20× magnification in the VTA. Double-label immunohistochemistry for Fos (green) and dopamine neuron marker TH (Blue; left panel) or GAD67 (red; right panel) in the VTA in methamphetamine-conditioned mice. We observed no co-localization of Fos with TH but approximately 70% of the Fos co-localized with GAD67. GAD67 is a marker for GABA neurons while TH is a marker for dopaminergic. Scale bar = 50 microns.

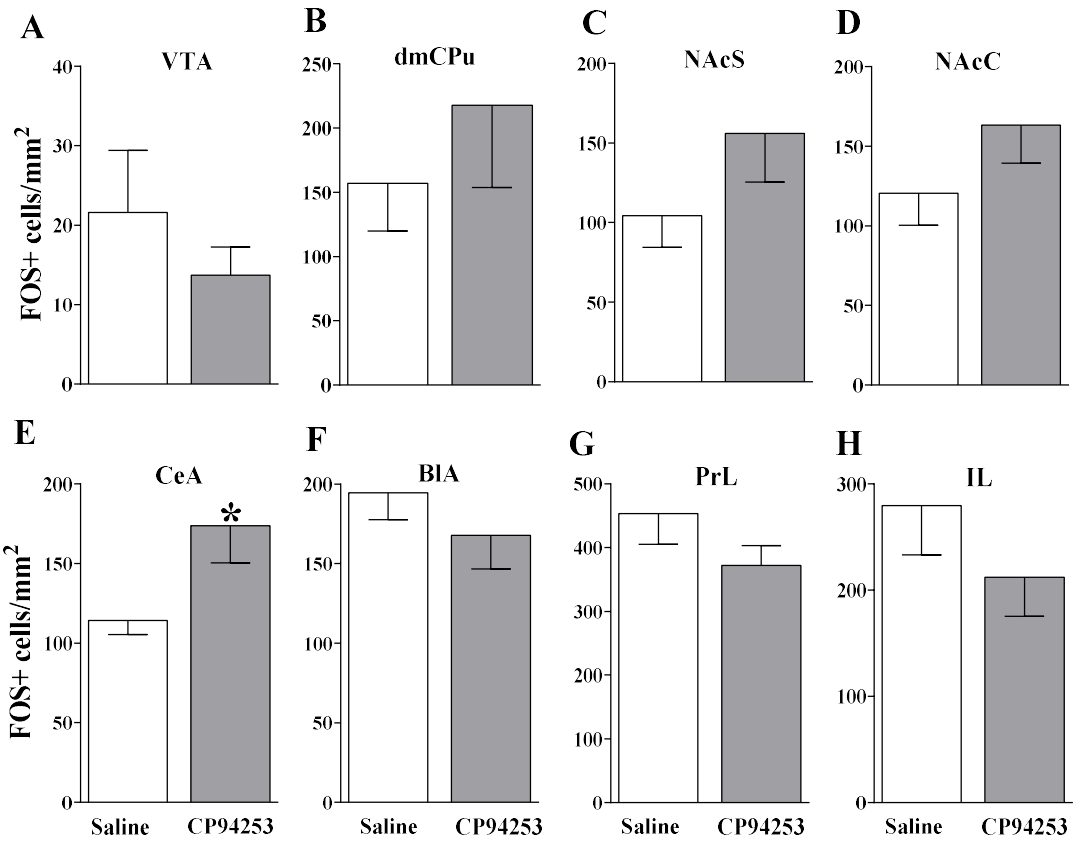

Figure 17. Mean+SEM of Fos positive cells per mm^2 for the (A) VTA, (B) dmCPu, (C) NAcS, (D) NAcC, (E) CeA, (F) BlA, (G) PrL, and the (H) IL (n=5-8/group). Mice were injected with saline (white bars) or 10 mg/kg, IP CP94253 (grey bars) 120 min prior to transcardial perfusion when brains were harvested for Fos protein immunohistochemistry. Asterisk (*) represents difference from Saline Group, t-tests $p<0.05$.

APPENDIX C

LIST OF ABBREVIATIONS

5-HT: serotonin
BlA: basolateral nucleus
CeA: central nucleus of the amygdala
CPP: conditioned place preference
CPu: caudate-putamen
DA: dopamine
DAPI: 4′,6-diamidino-2-phenylindole
dmCPu: dorsomedial caudate-putamen
FR: fixed ration
GABA: Gamma-Aminobutyric Acid
Glu: glutamate
IHC: immunohistochemistry
IL: infralimbic cortex
KO: knockout
PR: progressive ratio
PrL: prelimbic cortex
mPFC: medial prefrontal cortex
NAc: nucleus accumbens
NAcC: nucleus accumbens core
NAcS: nucleus accumbens shell
SA: self-administration
SUD: substance use disorder
TH: tyrosine hydroxylase
VR: variable ratio
VTA: ventral tegmental area

APPENDIX D

CURRICULUM VITAE

Curriculum Vitae

Taleen Der-Ghazarian

QUALIFICATIONS

- *Detailed statistical data analysis using Statistical Program for Social Sciences (SPSS) software*
  - *10 plus years' experience*
- *Data analysis using SQL, R, and Python*
  - *6 months experience*
- *Data collection*
  - *Manual*
  - *Software Generated*
  - *10 plus years' experience*
- *GraphPad Prism, Analyze and graphing software*
  - *10 years' experience*
- *Extensive ability to use Word, Excel, PowerPoint*
  - *18 years' experience*
- *Experience with Adobe illustrator and Adobe Photoshop*
  - *2 years' experience*
- *Collaborations*
  - *7 years' experience – Interdepartmental*
  - *4 years' experience – Medical Setting*
- *Mentoring Students*
  - *10 plus years' experience*
- *Team Management and Lab organization*
  - *10 plus years' experience*
- *Conference travel planning and organization*
  - *10 years' experience*
- *Writing and implementing IACUC and IBC protocols*
  - *7 years' experience*
- *Implementing Environmental, Health & Safety protocols (EH&S)*
  - *7 years' experience*
- *Writing and implementing IRB protocols*
  - *2 years' experience*
- *Online Course via Coursera: Teaching online*
  - May 2017, 5 week course
- *Online Course via Coursera: Teaching Science at a University*
  - May 2017, 5 week course

EDUCATION

- Neuroscience PhD
  Arizona State University, 2018
  - Under the supervision of Dr. Janet Neisewander
  - Graduate GPA: 4.0

- General Experimental Psychology MA, 2012

California State University, San Bernardino
  - Emphasis: Behavioral Neuroscience
  - Under the supervision of Dr. Sanders McDougall and Dr. Cynthia Crawford
  - Graduate GPA: 4.0
  - DIDARP fellow, Sally Casanova Scholar

- Biological Psychology BA, 2007
  California State University, San Bernardino
  - Psi Chi: National Honor Society in Psychology
  - Graduated with honors

RESEARCH, DATA ANALYSIS, TEAM MANAGEMENT EXPERIENCE

- Arizona State University, Research Assistant: 08/2011 - Present
  - School of Life Sciences, Neuroscience, Neuropharmacology of Drug Abuse Laboratory, Arizona State University, Tempe
    - I was responsible for designing the experiments I am conducting. This involves extensive literature reviews via the search engine Pubmed to design and execute a study using paradigms consistent to the literature in my field of study (drug abuse).
    - I collect and manage my experimental data within an SPSS database. Within SPSS statistical analysis on variables of interest is conducted.
    - The analyzed data is graphed using Prism and presented at conferences, seminars on campus, as well as publication in manuscript format.
    - Interdepartmental Collaboration with labs in the School of Life Sciences and as well the Psychology program in the School of Social and Behavioral Sciences.
    - Collaboration with St. Joseph's Hospital, Barrow Neurological Institute. We first collected preliminary data to write a collaborative RO1 grant which received 4 years of funding. I managed the collaborative experiments we conducted during the 4 year span of the grant.
    - Mentorship of undergraduate research assistants: Mentor and teach students to conduct experiments as well as present the data at lab meetings (see "Students Mentored" below).
    - Mentorship of Barrett Honor College students: Mentor and teach students to design experiments, execute those experiments, and write a thesis. These students form a thesis committee and present their prospectus as well as their final thesis (see "Students Mentored" below).
    - Team Management is crucial for the lab to operate in an orderly fashion. I typically manage 4-6 assistants which requires their involvement in experiments, participation in data and lab meetings, and the maintenance and cleaning of the lab.
    - Organize conference travel for the lab (i.e., hotel and transportation)
- Research Assistant and Lab Manager, California State University, San Bernardino: 03/2007 – 09/2011

- Department of Psychology, Neuropharmacology and Developmental Psychopharmacology Laboratories, California State University, San Bernardino
  - Similar to above with the following additions:
  - Managing and coordinating of behavioral experiments in the context of time and space availability. I was responsible for the scheduling of the behavioral experiments of all graduate students and played an overall supervisory role of 2 laboratories
  - Managing the breeding colony which involved reporting of the census to my PI daily, assigning of subjects to particular experiments, as well as following protocols implemented by IACUC for animal safety.

TEACHING EXPERIENCE

- Addiction and Recovery, Psych 334 (*Spring 2018*): Research and theories related to the psychological, behavioral and physiological basis of addiction and recovery. A variety of common addictive disorders will be considered including eating, smoking, gambling, work, sex and drugs. **Learning Objectives:** 1) To introduce students to the topic of Addiction & recovery, 2) to familiarize students with some of the theoretical and practical application aspects of this area, and 3) to provide students with enough information for them to be able to expand your investigation of, and/or involvement with, this area of psychology, should they choose to do so.
  - Lecture Professor
  - Lecture exams
  - In-class discussion/activities centered around an aspect of addiction.

- Behavioral Neuroscience, Psych 442 (*Winter 2018*): Intensive review of the neural mechanisms underlying behavior. Considerable emphasis is placed on sensory, motor, and homeostatic functioning. Higher-order functioning, including learning and memory, will also be covered. **Learning Objectives:** The goal of this class is to build a strong base of general knowledge in behavioral neuroscience, and to prepare students for graduate level study. The information covered will primarily review the basic principles of the central nervous system.
  - Lecture Professor
  - Tests and Quizzes on lecture material for the first 5 weeks of the quarter
  - Class Presentations: 50% of class time is dedicated to students presenting a chapter from the textbook.
  - Research Paper: Based off the topic of their chosen chapter presentations, students find primarily research articles and incorporate them into a reaction paper. The ***first*** section is the *Summary Section* where the main points of each article are presented. The ***second*** section is the *Evaluation Section* where the overall quality and impact of the articles to the topic of interest is judged. Students assess whether the data support the author's conclusions, whether the authors rejected or supported the experimental hypotheses presented in the Introduction, and the importance and relevance of the research project as it pertains to what is covered in the book chapter.

- Introduction to Experimental Psychology Writing Lab, Psych 311 (Winter 2018; *Spring 2018; Summer 2018*): Design and execution of psychological research. Four hours lecture and six hours laboratory. **Learning Objectives:** The primary objective of this lab class is to allow students the opportunity to experience the responsibilities involved in planning, conducting, and analyzing a psychological experiment. In addition, they will learn how to write a manuscript for publication of a psychological experiment. The process of doing research is vital, not the topic covered. The goal is to provide students with the fundamental skills needed to conduct and evaluate psychological research and to gain an understanding of the Scientific Method through hands-on experience.
    - Lecture Professor
    - Teach about Ethics in Research, APA Style writing, what plagiarism is and how to avoid it.
    - Conducted 2 experiments with the class and teach them experimental methodology and design.
    - Guide students to write manuscripts on the conducted experiments with all required section for a journal publication.

- Anatomy & Physiology, BIO 201 (*Fall 2017; Spring 2018; Summer 2018*): Studies the structure and function of the human body. Topics include cells, tissues, integumentary system, skeletal system, muscular system, and nervous system.
    - Online Lab Teaching Assistant
    - Grading quizzes
    - Grading lab practicals
    - Grading lab reports
    - Textbook publisher: McGraw-Hill
    - Connect: Digital teaching and learning environment
    - Experience teaching an online-only course

- Anatomy & Physiology, BIO 202 (*Fall 2017; Spring 2018; Summer 2018*): Studies the structure and function of the human body. Topics include cardiovascular, respiratory, lymphatic/immune, endocrine, renal, digestive, and reproductive systems.
    - Online Lab Teaching Assistant
    - Grading quizzes
    - Grading lab practicals
    - Grading lab reports
    - Textbook publisher: McGraw-Hill
    - Connect: Digital teaching and learning environment
    - Experience teaching an online-only course

- Animal Physiology, BIO 360 (*Spring 2016*): Principles and mechanisms of physiological regulation in animals, with a focus on humans.
    - Lecture teaching assistant

- Developing test questions
- Grading
- Blackboard Maintenance
- Mastering A&P: Digital teaching and learning environment
  - Question generating and grading
- Turning Point in class clicker question management
- Meeting with students during office hours

PROFESSIONAL DEVELOPMENT AND OUTREACH

- Served as a judge to assess travel, interview, internship, and research grants
  - 2011-2018
  - Monthly review of applications submitted by graduate students throughout all departments at ASU
- GAINS (Graduate Association of Interdisciplinary Neuroscience Students) – Held the officer position of Treasurer: 2013, 2014, 2015
  - Hosting Brain fairs at elementary schools throughout the Phoenix metro area
    - Work with children of various ages to teach them about brain structures and function.
  - Organized "The Brain & You: Neuroscience at ASU" symposium; Poster session and guest speaker panel discussing:
    - 'Career Options Outside Academia': 2014, 2015
    - 'The Next Step: Job Applications and Interviews': 2016

LABORATORY SKILLS

- Unlearned behavior assessment
  - Locomotor Assessment: activity monitoring chambers (Coulbourn Instruments)
  - Rotorod
  - Plus maze
  - Tail flick
  - Hot plate
  - Stereotypy scale
  - Plus maze
- Learned behavior assessment
  - Sucrose reinforced bar pressing (Coulbourn Instruments)
  - Sucrose water preference
  - Condition place preference paradigm
  - Drug self-administration
  - Morris water maze
- AAV Viral infusions (DREADDs) in NAc shell and core
- Electroconvulsive Shock (ECS)
- Injections
  - Intraperitoneal

    - Subcutaneous
    - Intravenous
- Surgical Skills
    - Small animal surgery
    - Operation of stereotaxic device
    - Coordinate verification
- Microdialysis
    - Unilateral cannula implantation (caudate-putamen)
    - Operation and maintenance of microdialysis equipment (i.e., syringe pumps, fraction collector, tubing)
- Microinjection
    - Bilateral cannula implantation (caudate-putamen, ventrolateral striatum, nucleus accumbens, medial prefrontal cortex, BLA, CEA)
    - Unilateral cannula implantation (lateral ventricles)
    - Precise microinjection procedure via Hamilton Syringes
    - Operation and maintenance of microinjection equipment (i.e., microinfusion syringes, syringe pumps, injectors)
- Tissue Preparation
    - Homogenate Ligand Binding Assay
    - Dopamine HPLC
    - PKA Assay
- Autoradiography
- Fluorescence immunohistochemistry
- Confocal Microscopy
- Cardiac Perfusion
- Brain Extraction
- Tissue Sectioning
    - Cryostat
    - Vibrotome
- Behavior assessment software
    - Truscan
    - Graphic State
    - Top Scan
- Managed breeding colony
- Makerbot2 3D printer Operation
- Laser Etching and Rotary

RESEARCH TOOLS & SKILLS

- *Data collection*
    - *Manual*
    - *Software Generated*
- *Detailed statistical data analysis using Statistical Program for Social Sciences (SPSS) software*
    - *9 plus years' experience*
- *Extensive ability to use Word, Excel, PowerPoint*
    - *16 years' experience*

- *Managed breeding colony*
    - *2007-2011*
- *GraphPad Prism, Analyze and graphing software*
    - *9 years' experience*
- *Experience with Adobe illustrator and Adobe Photoshop*
    - *2 years' experience*

STUDENTS MENTORED

- Cynthia Britt (Psychology undergraduate/graduate student)
- Fausto Varela (MIDARP)
- Tyler Stickney (MIDARP)
- Joseph Valentine (MIDARP)
- Joseph Pipkin (MIDARP)
- Olga Kozanian (Psychology graduate student)
- Ryan Lee (MARC)
- Leslie Amodeo (Psychology undergraduate/graduate)
- Alexandria Pothier (Psychology undergraduate/graduate)
- Arnold Gutierrez (Psychology undergraduate/graduate/MIDARP)
- Crystal Widarma (Biology/Chemistry undergraduate)
- Krystal Whittenberg (Psychology undergraduate)
- Kevin Castellanos (MARC)
- Alena Mohd-Yusof (Psychology undergraduate/graduate)
- Jellesa Johnson (MARC)
- Rebecca Mirando (Undergraduate)
- Kael Dai (Undergraduate)
- Sam Brunwasser (Barrett's Honors Collage/undergraduate)
- Kathryn Stefanko (Undergraduate)
- Pooja Viswanath (Barrett's Honors Collage/undergraduate)
- Vanessa Piscoya (Post baccalaureate)
- Sean Noudali (Undergraduate)
- Delaram Charmchi (Undergraduate)
- Aysha Mahmud (Barrett's Honors Collage/undergraduate)
- Samantha Scott (PREP Program, Neuroscience MA)
- Tanessa Call (Neuroscience PhD Rotation)

CONFERENCE PRESENTATIONS

- **Der-Ghazarian T**, Charmchi D, Noudali S, Mahmud A, Neisewander J (2017) Effects of a 5-HT_{1B} receptor agonist on the acquisition and expression of methamphetamine-conditioned place preference in C57BL/6 mice. Paper will be presented at the annual meeting of the Society for Neuroscience, Washington DC.
- **Der-Ghazarian T,** Brunwasser S, Dai K, Stefanco K, Call T, Scott S, Noudali S, Garcia R, Neisewander J (2016) Effects of a 5-HT_{1B} receptor agonist on locomotion and reinstatement of cocaine-conditioned place preference after abstinence from repeated injections in mice. Paper presented at the annual meeting of the Society for Neuroscience, San Diego and International Society for Serotonin Research Meeting, Seattle WA.

- **Der-Ghazarian T**, Gao M, Wu J, and Neisewander J (2015) 5-HT_{1B} receptor agonism has different effects on cocaine-induced locomotion and dopamine neuron activity in the VTA depending on time of testing after a repeated injection regimen in mice. Paper presented at American College of Neuropsychopjarmacology, Florida.
- **Der-Ghazarian T**, Brunwasser S, Dai K, Pentkowski J, Neisewander J (2014) Effects of the 5-HT_{1B}R agonist CP94253 on cocaine-induced locomotion before and after abstinence from repeated cocaine administration in C57BL/6 mice. Paper presented at the annual meeting of the Society for Neuroscience, Washington DC and The Brain and You: Neuroscience at ASU, Arizona.
- **Der-Ghazarian T**, Pockros L, Mirando R, Brunwasser S, Pentkowski J, Neisewander J (2013) 5-HT_{2A}R antagonism and 5-HT_{2C}R stimulation attenuates hyperlocomotion produced by intra-striatal cocaine infusions. Paper presented at the annual meeting of the Society for Neuroscience, San Diego and The Brain and You: Neuroscience at ASU, Arizona.
- McDougall SA, Pipkin JA, **Der-Ghazarian T**, Cortez AM, Gutierrez A, Lee RJ, Carbajal SM, Shaddox JL, Crawford CA (2013) Age-dependent differences in the persistence of cocaine-induced conditioned activity in adult and young rats: regional differences in Fos immunoreactivity. Paper presented at the annual meeting of the International Behavioral Neuroscience Society, Dublin, Ireland.
- **Der-Ghazarian T**, Varela FA, Lee R, Charntikov S, McDougall SA (2012) Repeated aripiprazole treatment causes receptor supersensitivity in young rats. Paper presented at the annual meeting of the Society for Neuroscience, New Orleans.
- Pockros LA, **Der-Ghazarian T**, Pentkowski NS, Conway SM, Zwick K, Harder BG, Neisewander JL (2012) Effects of serotonin 2C receptor stimulation in the BLA on reinstatement of cocaine-seeking behavior and anxiety-like behavior on the elevated plus maze. Paper presented at the annual meeting of the Society for Neuroscience, New Orleans.
- Valentine JM, Britt CE, Herbert MS, **Der-Ghazarian T**, Varela FA, Kozanian OO, Whittenburg KL, Pipkin JA, Johnson JD, Humphrey DE, Crawford (2012) Early methylphenidate exposure alters kappa opioid receptor mediated antinociception and body temperature. Paper presented at the annual meeting of the Society for Neuroscience, New Orleans.
- Pentkowski NS, Harder B, Brunwasser S, Yanamandra K, Bastle R, **Der-Ghazarian T**, Adams M, Alba J, Neisewander JL (2012) The effects of 5-HT_{1B} receptors on motivation for cocaine vary depending on the length of abstinence. Paper presented at the annual meeting of the Society for Neuroscience, New Orleans.
- Herbert MS, Valentine JM, Varela FA, **Der-Ghazarian T**, Kozanian OO, Amodeo LR, Whittenburg K, Lee RJ, Bradley LA, Crawford, CA (2012) The kappa opioid system is sensitized after early methylphenitdate exposure in the rat.

Paper presented at the annual meeting of the International Associated for the Study of Pain, Milan, Italy.

- Pentkowski NS, Harder B, Brunwasser S, Bastle R, **Der-Ghazarian T**, Adams M, Alba J, Neisewander JL (2012) Stimulation of serotonin-1B receptors attenuates cocaine-abuse related behaviors following protracted withdrawal. Paper presented at the annual meeting of The College on Problems of Drug Dependence, Palm Springs.
- **Der-Ghazarian T,** Guitierrez A, Widarma CB, Varela FA, McDougall SA (2011) Paradoxical behavioral effects of DA receptor inactivation in young rats: Receptor specificity. Paper presented at the annual meeting of the Society for Neuroscience, Washington DC.
- **Der-Ghazarian T**, Pipkin JA, Gutierrez A, Carbajal SM, Cortez AM, McDougall SA (2011) Persistence of one-trial cocaine-induced conditioned activity in young and adult rats. Paper presented at the meeting of the annual Society for Neuroscience, Washington DC.
- Valentine JM, Herbert MS, **Der-Ghazarian T**, Horn LR, Kozanian OO, Varela FA, Whittenburg K, Crawford, CA (2011) Effects of early methylphenidate exposure on DAMGO- and morphine-induced antinociception. Paper presented at the annual meeting of the Society for Neuroscience, Washington DC.
- **Der-Ghazarian T**, Charntikov S, Varela FA, McDougall SA (2010) Effects of repeated aripiprazole treatment on the amphetamine-induced locomotor activity and stereotypy of preweanling rats. Paper presented at the annual meeting of the Society for Neuroscience, San Diego.
- **Der-Ghazarian T**, Britt CE, Varela FA, Crawford CA, McDougall SA (2010) Long-term effects of postnatal manganese exposure on the expression of D2S and D2L receptor isoforms: impact on PKA activity, p-ERK, and p-AKT levels. Paper presented at the annual meeting of the Society for Neuroscience, San Diego.
- **Der-Ghazarian T**, Horn LR, Herbert MS, Gutierrez A, Widarma CB, Charntikov S, McDougall SA (2010) Paradoxical behavioral effects of DA receptor inactivation in young rats: role of the dorsal caudate-putamen. Paper presented at the meeting of the annual Society for Neuroscience, San Diego.
- Herbert MS, Charntikov S, **Der-Ghazarian T**, Horn LR, Widarma CB, McDougall SA (2010) Effects of D1 and D2 receptor stimulation in the dorsal caudate-putamen of preweanling rats: impact on locomotor activity and stereotypy. Paper presented at the annual meeting of the Society for Neuroscience, San Diego.
- Palmer AG, Cortez AM, Herbert MS, **Der-Ghazarian T**, Britt CE, Castellanos KA, McDougall SA (2010) Temporal factors affecting the one-trial cocaine-induced behavioral sensitization of young rats. Paper presented at the annual meeting of the Society for Neuroscience, San Diego.

- Stickney TC, Kozanian OO, Britt CE, **Der-Ghazarian TS**, Crawford CA (2010) Effects of preweanling methylphenidate exposure on sucrose preference and novelty-induced locomotor activity. Paper presented at the annual meeting of the Society for Neuroscience, San Diego.
- **Der-Ghazarian T**, Britt CE, Varela F, Roper AJ, Crawford CA (2010) Effects of preweanling, preadolescent, and adolescent methylphenidate treatment on morphine-induced conditioned place preference. Paper presented at the annual meeting of The College on Problems of Drug Dependence, Scottsdale.
- Taylor C, **Der-Ghazarian T**, Kaufman J (2010) The influence of age on popularity of performers between genders. Paper presented at the annual meeting of the Western Psychological Association, Cancun, Mexico.
- **Der-Ghazarian T**, Britt C, Varela F, Roper A, Mobley R, Crawford C (2009) Effects of preweanling methylphenidate treatment on novelty-induced CPP. Paper presented at the annual meeting of the Society for Neuroscience, Chicago.
- Herbert M, **Der-Ghazarian T**, Palmer A, McDougall S (2009) One-trial cocaine-induced behavioral sensitization in the young rat: Effects of injection procedures and drug cues. Paper presented at the annual meeting of the Society for Neuroscience, Chicago.
- **Der-Ghazarian T**, Martinez C, Koshino H (2009) Individual differences in working memory capacity and visual selective attention. Paper presented at the annual meeting of the American Psychological Association, Toronto, Canada.
- **Der-Ghazarian T**, Martinez C, Varela F, Crawford C, McDougall S (2008) Persistent effects of postnatal manganese exposure on protein kinase A (PKA) activity in the striatum, medial prefrontal cortex, and hippocampus of adults rats. Paper presented at the annual meeting of the Society for Neuroscience, Washington DC.

PUBLICATIONS

- **Der-Ghazarian T**, Call T, Scott S, Brunswasser S, Dai K, Noudali S, Pentkowski N, Neisewander J (2017) Effects of a 5-HT1B Receptor Agonist on Locomotion and Reinstatement of
Cocaine-Conditioned Place Preference After Abstinence from Repeated Injections in Mice**.** *Frontiers in Systems Neuroscience* 11:73
- McDougall SA, Pipkin JA, **Der-Ghazarian T**, Cortez AM, Gutierrez A, Lee RJ, Carbajal S, Mohd-Yusof A (2014) Age-dependent differences in the persistence of psychostimulant-induced conditioned activity in rats: effects of a single environment-cocaine pairing. *Behavioral Pharmacology* 25:695-704
- Pockros-Burgess LA, Pentkowski NS, **Der-Ghazarian T**, Neisewander JL (2014). Effects of the 5-HT2C receptor agonist CP809101 in the amygdala on reinstatement of cocaine-seeking behavior and anxiety-like behavior. *Journal of Neuropsychopharmacology* 17:1751-1762

- Pentkowski NS, Harder BG, Brunwasser SJ, Bastle RM, Peartree NS, Yanamandra K, Adams MD, **Der-Ghazarian T**, Neisewander JL (2014). Pharmacological evidence for an abstinence-induced switch in 5-HT_{1B} receptor modulation of cocaine self-administration and cocaine-seeking behavior. *ACS Chemical Neuroscience* 5:168-176
- **Der-Ghazarian T**, Widarma CB, Gutierrez A, Amodeo LR, Valentine JM, Humphrey DE, Gonzalez AE, Crawford CA, McDougall SA (2014) Behavioral effects of dopamine receptor inactivation in the caudate-putamen of preweanling rats: role of the D2 receptor. *Pschopharmacology* 1:651-662
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- **Der-Ghazarian T**, Gutierrez A, Varela FA, Herbert MS, Amodeo LR, and Charntikov S, Crawford CA, McDougall SA (2012) Dopamine receptor inactivation in the caudate-putamen differentially affects the behavior of preweanling and adult rats. *Neuroscience* 226C:427-440
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- McDougall SA, **Der-Ghazarian T**, Britt CE, Varela FA, Crawford CA (2011) Postnatal manganese exposure alters the expression of D2L and D2S receptor isoforms: Relationship to PKA activity and Akt Levels. *Synapse* 65(7):583-591
- Cortez AM, Charntikov S, **Der-Ghazarian T**, Horn LR, Crawford CA, McDougall SA (2010) Age-dependent effects of kappa-opioid receptor stimulation on cocaine-induced stereotyped behaviors and dopamine overflow in the caudate-putamen: an in vivo microdialysis study. *Neuroscience* 169(1):203-213
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- McDougall SA, Reichel CM, Farley CM, Flesher MM, **Der-Ghazarian T**, Cortez AM, Wacan JJ, Martinez CE, Varela FA, Butt AE, Crawford CA (2008) Postnatal manganese exposure alters dopamine transporter function in adult rats: Potential impact on nonassociative and associative processes. *Neuroscience* 154(2):848-860

MANUSCRIPTS IN PREPARATION

- **Der-Ghazarian T**, Pockros L, Pentkowski N, Mirando R, Brunwasser S, Neisewander J (2018) 5-HT_{2A}R antagonism and 5-HT_{2C}R stimulation attenuates intraCPu-induced cocaine hyperlocomotion.
- **Der-Ghazarian** T, Noudali S, Charmchi D, Scott S, Neisewander J (2018) 5-HT_{1B} receptor agonist attenuates expression of methamphetamine-conditioned place preference and reverses Fos expression changes in male c57 mice.

SUBMITTED MANUSCRIPTS

- Chen D, Gao F, MA X, Yang K, Gao M, Chang Y, **Der-Ghazarian T**, Neisewander J, Su Q, Wu J (2017) Cocaine directly inhibits α6-containing nicotinic acetylcoline receptor-mediated currents in human SH-ER1 cells. *October 2017*

AWARDS and SCHOLARSHIPS

- Research and Travel Award, GPSA:
  - 2011 ($950), 2013 ($950), 2014 ($950), 2015 ($950), 2016 ($950), 2017($950)
- SOLS Department Travel Funding:
  - 2013 ($400), 2014 ($400), 2015 ($400), 2016 ($400), 2017 ($400)
- Graduate College Travel Award:
  - 2017 ($500)
- Arizona State University/Barrow Neurological Association Inter-Institutional Graduate Fellowship: 2013-2014
  - Tuition coverage and $24,000 annual stipend
- Doctoral Recruiting Fellowship, ASU: 2011-2012
  - Tuition coverage and $24,000 annual stipend
- Graduate Equity Fellowship, CSUSB:
  - 2010-2011 ($3000), 2009-2010 ($3000)
- California Pre-Doctoral Fellowship, Sally Casanova Scholar, CSUSB:
  - 2010-2011 ($2000)
- MIDARP Scholar, CSUSB:
  - 2010-2011, 2009-2010, tuition reimbursement and monthly $1000 stipend
- First Place Award: CSUSB Research Conference Day, 2010

- CSUSB Research and Travel Award, ASI:
    - 2012 (1 award), 2011 (1 award), 2010 (3 awards), 2009 (3 awards), 2008 (2 awards), 2007 (1 award)

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